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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Pierre CHARNEAU et al.	)	Prior Group Art Unit: 1648
	)	
Serial No.: Not assigned (Based on Serial No.: 08/817,441)	)	Prior Examiner: R. Budens
	)	
Filed: December 27, 2001	)	

For: NUCLEOTIDE SEQUENCES OF HIV-1 TYPE (OR SUBTYPE) O RETROVIRUS ANTIGENS

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to examination of this application, please amend this application as follows:

IN THE SPECIFICATION:

On page 5, line 26, insert the following description of the drawings:

--BRIEF DESCRIPTION OF THE DRAWINGS

This invention will be more fully described with reference to the drawings in which:

Figures 1A and B depict RT activity and p24 levels.

Figure 2 is a Western blot analysis of a series of serum samples.

Figures 3A-D show an amino acid alignment of the HIV-1VAU and HIV-1LAI envelope sequences (SEQ ID NO:102 and SEQ ID NO:103), including an alignment of

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HIV-1VAU and HIV-1LAI gp120 sequences (SEQ ID NO:46 and SEQ ID NO:48) and HIV-1VAU and HIV-1LAI gp41 sequences (SEQ ID NO:47 and SEQ ID NO:49).

Figure 4 is a multiple alignment of the immunodominant peptides in the extracellular segment of the transmembrane envelope glycoprotein of various HIV-1 isolates (SEQ ID NOs:50-62).

Figures 5A-B contain branches of a phylogenetic tree.

Figure 6 is the DNA sequence of HIV-1VAU env gene (SEQ ID NO:63).

Figure 7 is the DNA sequence of HIV-1VAU virus integrase gene (SEQ ID NO:64).

Figures 8A-C contain a comparison of the GAG amino acid sequence of various HIV-1 strains (SEQ ID NOs:65-70).

Figure 9A is a multiple alignment of the V3 loop of gp120 (SEQ ID NOs:71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, and 93).

Figure 9B is a multiple alignment of the immunodominant region of gp41 (SEQ ID NOs:72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, and 94).

Figure 10A is a 513 base pair DNA sequence of the GAG region of HIV-1DUR (SEQ ID NO:95).

Figure 10B is the corresponding 171 amino acid sequence of Figure 10A (SEQ ID NO:96).

Figure 11A is a 525 base pair DNA sequence of the V3 loop region of gp120 of HIV-1DUR (SEQ ID NO:97).

Figure 11B is the corresponding 175 amino acid sequence of Figure 11A (SEQ ID NO:98).

Figure 12A is a 312 base pair DNA sequence of the immunodominant region of gp41 of HIV-1DUR (SEQ ID NO:99).

Figure 12B is the corresponding 104 amino acid sequence of Figure 12A (SEQ ID NO:100).

Figure 13A shows specific primer sequences for HIV-1 type O (SEQ ID NOs:42-45).

Figure 13B shows the corresponding positions of the primers of Figure 13A in different HIV type O strains.

Figures 14A-B are serological correlation results.

Figures 15A-C are nucleotide comparisons of HIV sequences.

Figures 16A-C are protein comparisons of HIV sequences.

Figure 17 is a phylogenetic tree.

Figure 18A-B show an HPLC chromatogram of a VAU peptide.

Figure 19A-B show a mass spectroscopy analysis of a VAU peptide (SEQ ID NO:101).

Figures 20A-C contain immunoreactivity data.

Figures 21A-C contain immunoreactivity data.--

Page 8, beginning at line 26 through page 9, line 2, replace this paragraph with the following text:

-- Two weeks after coculturing the patient's CD8-depleted, PHA-stimulated PBMCs with similar cells from a healthy donor, the production of virus was detected in the form of an RT activity peak in the culture supernatant. This virus could then be subjected to serial passages on CD8-depleted, PHA-stimulated normal PBMCs. Figure 1A represents the production of HIV-$1_{(VAU)}$ in infected PBMC culture supernatants, checked by RT assay (filled circles) and HIV-1 p24 antigen capture ELISA (empty circles). The concentration of HIV-1 p24 is expressed in ng/ml and the RT activity in

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cpm/μl. In Figure 1B, the same experiment was carried out with a standard primary HIV-1 isolate from an AIDS patient. --

Page 11, beginning at line 14 through line 20, replace this paragraph with the following text:

-- The invention relates to any variant of the nucleic acid sequences of the HIV-$1_{(VAU)}$ virus or of any group O equivalent virus, containing structural proteins which have the same immunological properties as the structural proteins coded for by the env gene comprising the sequence described in Figure 6 and called "vau", also designated by SEQ ID NO:63. --

Page 11, beginning at line 33 through page 12, line 11, replace this paragraph with the following text:

-- The invention relates to the DNAs or DNA fragments, more particularly cloned DNAs and DNA fragments, obtained from RNA, cDNA or primers which can be used in PCR, or other gene amplification methods, derived from the HIV-$1_{(VAU)}$ retrovirus RNA or DNA. The invention relates more particularly to all the equivalent DNAs, especially to any DNA having sequence homologies with the HIV-$1_{(VAU)}$ DNA, in particular with the sequence coding for the *env* region of the $HIV_{(VAU)}$ strain comprising the sequence corresponding to SEQ ID NO:63 represented in Figure 6 and called "vau". The homology with HIV-1 group M is at least equal to 50%, preferably to 70% and still more advantageously to about 90%. Generally, the invention relates to any equivalent DNA (or RNA) capable of hybridizing with the DNA or RNA of a group O HIV-1 retrovirus. --

Page 12, beginning at line 14 through line 18, replace this paragraph with the following text:

--The invention also relates to the HIV-$1_{(VAU)}$ integrase gene comprising the sequence identified by the same SEQ ID NO:64 or hybridizing with SEQ ID NO:64. The invention also relates to the RNAs corresponding to the DNA described above. --

Page 13, beginning at line 19 through page 14, line 11, replace this paragraph with the following text:

-- The resulting amplification product was cloned into a pBluescript vector, generating the clone ph4, deposited at the Collection Nationale des Cultures de Micro-organismes (CNCM), Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15 France, on 20 October 1994 under No. I-1486, which was subsequently used as a probe to screen a lambda library of low molecular weight DNA, which was digested with EcoRI and was obtained from cells infected with HIV-$1_{(VAU)}$. Briefly, the PBMCs infected

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with HIV-$1_{(VAU)}$ were cocultured for 24 hours with new PBMCs stimulated with PHA and depleted of $CD8^+$ cells, after which a high cytopathic effect (CPE) was visible. The low molecular weight DNA was then extracted according to the Hirt method (Hirt 1967), and digested with the enzyme EcoRI. A previous Southern-blot analysis of this DNA had indeed shown that the HIV-$1_{(VAU)}$ genome contained only one EcoRI site, permitting the cloning of nonintegrated circular DNA species representing the entire viral genome. The resulting digestion product was subjected to agarose gel electrophoresis, and the population of DNA fragments of approximately 8-12 kb in size was purified and ligated to EcoRI-digested lambda Zap DNA (Stratagene). After encapsidation, plating and screening by hybridization with ^{32}P-labeled ph4 DNA, a clone, λH34, was identified as being positive, and amplified. The EcoRI insert was purified, sonicated, and cloned by the "shotgun" technique into the phosphatase-treated vector M13mp18 digested with the enzyme SmaI. One hundred and fifty of the clones obtained were sequenced in a 373A DNA sequencer (Applied Biosystems), and the resulting sequences were assembled into a single sequence using the Wisconsin GCG DNA analysis package. --

Page 14, beginning at line 24 through line 36, replace this paragraph with the following text:

-- The PCR amplification was carried out in 35 thermal cycles at 92°C for 15 seconds, 52°C for 1 minute, 60°C for 2 minutes and 72°C for 2 minutes. The resulting amplification product, of 3.5 kb in size, was cloned into the M13mp18 vector and sequenced by successive reactions, first using the M13 universal sequencing primer, and then the primers deduced from the upstream sequences. Analysis of the nucleotide and peptide sequences was carried out using the Wisconsin GCG DNA analysis package. The HIV-$1_{(VAU)}$ *env* gene codes for 877 amino acids in total, including the signal peptide. The nucleotide sequence of the HIV-$1_{(VAU)}$ *env* gene corresponds to SEQ ID NO:63 (see Figure 3). --

Page 15, beginning at line 29 through line 37, replace this paragraph with the following text:

-- Particularly advantageous are the probes which, when hybridized with HIV-1, give a strong reaction with HIVs belonging to group O and weak reaction with HIVs belonging to group M. By way of nonlimiting example, a probe constructed from the HIV-$1_{(VAU)}$ virus integrase gene sequence SEQ ID NO:64 gives, when it is hybridized with HIV-1 under hybridization conditions such as those described in Patent EP 178 978, a strong reaction with group O HIVs and a weak reaction with group M HIVs. --

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Page 17, beginning at line 13 through line 23, replace this paragraph with the following text:

-- The invention relates to an external envelope protein of the HIV-$1_{(VAU)}$ retrovirus encoded by the gene comprising the sequence corresponding to SEQ ID NO:63. According to a preferred embodiment of the invention, this protein is in addition characterized in that it comprises the amino acid sequence corresponding to SEQ ID NO:46 represented in Figure 3 and comprising amino acid residues 1 to 526. The subject of the invention is also any polypeptide or variant which is derived from said sequence having an epitope which may be recognized by the antibodies induced by the HIV-$1_{(VAU)}$ virus. --

Page 17, beginning at line 26 through line 31, replace this paragraph with the following text:

-- The subject of the invention is also an envelope transmembrane protein comprising the amino acid sequence SEQ ID NO:47 represented in Figure 3 between amino acid residues 527 and 877. This transmembrane protein is, within the scope of the invention, in glycosylated or nonglycoslated form. --

Page 19, beginning at line 7 through line 17, replace these paragraphs with the following text:

-- Preferred polypeptides of this region are, for example, those which contain the sequence CKNRLIC (SEQ ID NO:5) or correspond to this sequence. They may also be peptides or polypeptides corresponding to the sequence RLLALETFIQNWWLLNLWGCKNRLIC (SEQ ID NO:6) or comprising this sequence.

Another preferred peptide, identified below by the name "VAU peptide", corresponds to the following sequence or comprises this sequence or any part of this sequence capable of being recognized by antibodies directed against the HIV-$1_{(VAU)}$ retrovirus RARLLALETFIQNQQLLNLWGCKNRLICYTSVKWNKT (SEQ ID NO:7). --

Page 19, beginning at line 24 through line 31, replace this paragraph with the following text:

-- The present invention relates to a peptide obtained from the HIV-1-O DUR virus deposited on 23 February 1995 at the Collection Nationale des Cultures de Micro-organismes (CNCM), Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15 France, under the reference I-1542, or a peptide whose sequence is distinguished from that of the above by substitution, deletion or addition of amino acids, this separate peptide nevertheless retaining the antigenic characteristics of the above one. --

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Page 19, beginning at line 34 through page 21, line 9, replace these paragraphs with the following text:

-- Thus, a preferred peptide of the invention is a peptide containing at least 4 consecutive amino acids contained in the GAG sequence represented in Figure 8A-C or in an immunologically similar GAG sequence obtained from a variant of the HIV-1-O DUR virus, said immunologically similar sequence being recognized by antibodies which also specifically recognize at least one of the sequences AHPQQA (SEQ ID NO:8), LWTTRAGNP (SEQ ID NO:9) contained in the GAG sequence of Figure 8.

Preferably, this peptide consists of a peptide whose amino acid sequence is contained either in one of the following sequences:

SPRTLNAWVKAVEEKAFNPEIIPMFMALSEGA (1) (SEQ ID NO:10)
MLNAIGGHQGALQVLKEVIN (2) (SEQ ID NO:11)
GPLPPGQIREPTGSDIAGTTSTQQEQI (3) (SEQ ID NO:12)
IPVGDIYRKWIVLGLNKMVKMYSPVSILDI (4) (SEQ ID NO:13)
QGPKEPFRDYVDRFYKTKLAE (5) (SEQ ID NO:14)
AHPQQA (5a) (SEQ ID NO:8)
LWTTRAGNP (5b) (SEQ ID NO:9)

or in a corresponding immunologically similar sequence, this peptide containing at least four consecutive amino acids of one of said sequences.

Preferably also, this peptide consists of a peptide whose amino acid sequence is contained either in one of the following sequences:

SPRTLNAWVK (6) (SEQ ID NO:15)
GSDIAGTTST (7) (SEQ ID NO: 16)
QGPKEPFRDYVDRF (8) (SEQ ID NO: 17)

or in a corresponding immunologically similar sequence, this peptide containing at least four consecutive amino acids of one of said sequences.

Peptides which are particularly preferred in the present invention are the peptides containing:

-the amino acid sequence NPEI (9) (SEQ ID NO:18)
or
- the amino acid sequence AVEEKAFNPEIIPMFM (10) (SEQ ID NO:19), and

more particularly peptides whose amino acid sequence is contained, either in one of the following sequences:

IGGHQGALQ (23) (SEQ ID NO:20)
REPTGSDI (24) (SEQ ID NO:21)

or in a corresponding immunologically similar sequence, this peptide containing at least 4 consecutive amino acids of one of said sequences, as well as the peptide whose amino acid sequence is contained, in the following amino acid sequence:

IDEAADWD (25) (SEQ ID NO:22)

or in a corresponding immunologically similar sequence, this peptide containing at least four consecutive amino acids of said sequence. --

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Page 21, beginning at line 20 through line 33, replace this paragraph with the following text:

-- A peptide derived from the HIV-1-O DUR virus defined above also falls within the scope of the present invention, said peptide containing at least 4 consecutive amino acids of the V3 loop of gp120 represented in Figure 9A or of the corresponding immunologically similar sequence, obtained from a variant of the HIV-1-O DUR virus, said immunologically similar sequence being recognized by antibodies which also specifically recognize at least one of the sequences:

KEIKI (12) (SEQ ID NO:23)
EREGKGAN (13) (SEQ ID NO: 24)
CVRPGNNSVKEIKI (14) (SEQ ID NO:25)
QIEREGKGANSR (15) (SEQ ID NO:26). --

Page 21, beginning at line 35 through page 22, line 14, replace this paragraph with the following text:

-- This peptide preferably contains:

a) either the sequence CVRPGNNSVKEIKIGPMAWYSMQIEREGKGANSRTAFC (11) (SEQ ID NO: 27) or a part of this sequence which contains at least 4 amino acids

b) or an amino acid sequence which is separate from the sequence of a) in which one or more amino acids are replaced with one or more amino acids, with the proviso that the peptide retains its reactivity with an antiserum against the abovementioned peptide,

c) or an amino acid sequence which is separate from a) or b), in which one or more amino acids have been deleted or added, with the proviso that the peptide retains its reactivity with an antiserum against the peptide of a),

d) or a corresponding immunologically similar sequence or part of a sequence. --

Page 22, beginning at line 16 through page 23, line 5, replace these paragraphs with the following text:

-- Preferably also, this peptide contains either
the sequence KEIKI (12) (SEQ ID NO:23),
or
the sequence EREGKGAN (13) (SEQ ID NO:24),
or
the sequence GPMAWYSM (16) (SEQ ID NO:28).

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In a particularly preferred manner, a peptide as defined above contains the amino acid sequence CVRPGNNSVKEIKI (14) (SEQ ID NO:25) or the sequence QIEREGKGANSR (15) (SEQ ID NO:26).

A peptide derived from the HIV-1-O DUR virus as defined above also falls within the scope of the invention, said peptide containing at least 4 consecutive amino acids, whose entire sequence is contained in the sequence of the immunodominant region of gp41 represented in Figure 9B or in a corresponding immunologically similar sequence, obtained from a variant of the HIV-1-O DUR virus, said immunologically similar sequence being recognized by antibodies which also specifically recognize at least one of the following sequences:

RLLALETLMQNQQL (17) (SEQ ID NO:29),
LNLWGCRGKAICYTSVQWNETWG (18) (SEQ ID NO:30),
CRGKAI (19) (SEQ ID NO:31),
SVQWN (20) (SEQ ID NO:32),
RLLALETLMQNQQLLNLWGCRGKAICYTS (21) (SEQ ID NO:33),
QNQQLLNLWGCRGKAICYTSVQWN (22) (SEQ ID NO:34). --

Page 23, beginning at line 7 though line 27, replace this paragraph with the following text:

-- This peptide is preferably a peptide containing the sequence RLLALETLMQNQQL (17) (SEQ ID NO:29) or LNLWGCRGKAICYTSVQWNETWG (18) (SEQ ID NO:30) or part of this peptide (18) (SEQ ID NO:30) containing:

a) either the sequence CRGKAI (19) (SEQ ID NO:31) or the sequence SVQWN (20) (SEQ ID NO:32) in which Q is, where appropriate, replaced by a different amino acid, which is nevertheless also different from K, or the two sequences at the same time,

b) or an amino acid sequence which is separate from the sequence of a) in which one or more amino acids are replaced with two amino acids, with the proviso that the peptide retains its reactivity with an antiserum against the peptide of a),

c) or an amino acid sequence which is separate from a) or b), in which one or more amino acids have been deleted or added, with the proviso that the peptide retains its reactivity with an antiserum against the peptide of a),

d) or in a corresponding immunologically similar sequence or part of a sequence. --

Page 23, beginning at line 29 through page 24, line 4, replace this paragraph with the following text:

-- Preferably also, this peptide possesses one or the other of the following characteristics:

- its N-terminal sequence which contains at least 8 amino acids is not immunologically recognized by antibodies formed against the sequence RILAVERY

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(SEQ ID NO:35) contained in the immunodominant region of gp41 of the HIV-1-LAI strain.

- it is not recognized by antibodies formed against the peptide SGKLIC (SEQ ID NO:36) of the HIV-1-LAI strain.
- it contains either of the following two sequences:
RLLALETLMQNQQLLNLWGCRGKAICYTS (21) (SEQ ID NO:33)
QNQQLLNLWGCRGKAICYTSVQWN (22) (SEQ ID NO: 34). --

Page 25, beginning at line 9 through line 25, replace this paragraph with the following text:

-- The peptide is checked by HPLC and by mass spectrometry according to the electrospray technique (Figures 18A-B and Figures 19A-B) (FISON VG Trio 2000 spectrophotometer).

Fmoc: 9-Fluoroenylmethyloxycarbonyl
Pmc: 8-Methylpentane-6-sulfonylchroman
Trt: Tritryl
Boc: Tertbutyloxycarbonyl
tBU: tert butyl
DMF: Dimethylformamide
DIPCDI: Diisopropylcarbodiimide
HOBT: 1-Hydorxybenzotriazole
TF: Trifluoroacetic acid
Reagent K: Phenol/water/thioanisole/ethanedithiol/TFA: 2.5 ml/2.5 ml/2.5 ml/1.5 ml/41 ml
Comparison of the amino acid sequence of the HIV-$1_{(VAU)}$ envelope with the corresponding sequence of other HIV viruses. --

Page 26, beginning at line 31 through page 27, line 9, replace this paragraph with the following text:

-- The alignment of Figure 3 shows numerous regions of high divergence, with a few domains retained here and there. These retained regions correspond roughly to the domains also retained in the conventional HIV-1 isolates (Alizon et al. 1986, Benn et al. 1985). Among the divergent domains, the V3 loop, also called principal determinant of neutralization (Javaherian et al. 1990, Javaherian et al. 1989, Matsushita et al. 1988) is clearly one of the most divergent, although the two cysteines defining the loop are retained. The sequence of the cap of the loop, GPGRAF (SEQ ID NO:37) for HIV-1-LAI is GPMAWY (SEQ ID NO:38) in HIV-$1_{(VAU)}$. This unit of the cap is identical to that of the Cameroonian group O isolate $HIV_{(ANT70)}$ (Van den Heasevelde et al. 1994), but is different from that of the other group O isolate, HIV $_{(MVP5180)}$ (Gűrtler et al. 1994), for which the motif is GPMRWR (SEQ ID NO:39). --

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Page 30, beginning at line 30 through page 31, line 16, replace this paragraph with the following text:

-- Generally, the invention relates to any composition which can be used for the in vitro detection of the presence, in a biological fluid, especially from individuals who have been brought into contact with HIV-$1_{(VAU)}$, or with antibodies against at least one of the HIV-$1_{(VAU)}$ antigens. This composition can be applied to the selective diagnosis of infection by an HIV-1 group O by using diagnostic techniques such as those described in Patent Applications EP 84401834 and EP 87400,151,4. Within the context of the present invention, any constituent comprising antigenic determinants capable of being recognized by antibodies produced against HIV-$1_{(VAU)}$ is used, for example recombinant antigens or peptides or chemically synthesized peptides defined from the sequence of the HIV-$1_{(VAU)}$ envelope. In this regard, the invention relates more particularly to compositions containing at least one of the HIV-$1_{(VAU)}$ virus envelope proteins. There may be mentioned, by way of examples of compositions, those which contain proteins, glycoproteins or peptides from the envelope protein corresponding to the entire 590-620 region of the HIV-$1_{(VAU)}$ gp41 protein or to the parts of this region which are specific for HIV-$1_{(VAU)}$ such as the peptides -TFIQN- (SEQ ID NO:40) or -WGCKNR- (SEQ ID NO:41). --

Page 37, beginning at line 21, through page 38, line 13, replace this paragraph with the following text:

-- The experimental data collated in the two tables of Figures 20A-C and 21A-C show that:

a) the four sera taken from patients contaminated with the HIV-1 group (or subgroup) O virus are very reactive with the vau peptide;

b) the ten sera supposedly taken from patients contaminated with the HIV-1 group (or subgroup) O virus, among the 19 sera sent out by the Pasteur Institute of Yacoundé, are also highly reactive with this same peptide;

c) the sera (4 samples) taken from individuals contaminated with the HIV-1 subtype B virus (in the acute phase) are not reactive with the vau peptide;

d) the sera taken from asymptomatic blood donors (48 samples tested) are not reactive with the vau peptide; These experimental data, although limited (in view of the paucity of HIV-1 group (or subgroup O) antibody-positive samples), bear witness to the sensitivity and specificity of the peptide selected. --

Page 40, beginning at line 10 through line 16, replace this paragraph with the following text:

-- According to the present invention, the process of detection and discrimination between infection by an HIV-1 group (or subgroup) O retrovirus and an HIV-1 subgroup

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M retrovirus is characterized by placing serum, obtained from individuals subjected to an AIDS diagnostic test, in contact, in particular, with the peptide RILAVERY (SEQ ID NO:35). --

Page 45, beginning at line 19 through line 26, replace this paragraph with the following text:

-- Oligonucleotide primers also according to the invention have a sequence consisting of at least eight consecutive nucleotides of the following nucleotide sequences:

ATT CCA ATA CAC TAT TGT GCT CCA-3' (SEQ ID NO:42)
AAA GAA TTC TCC ATG ACT GTT AAA-3' (SEQ ID NO:43)
GGT ATA GTG CAA CAG CAG GAC AAC-3' (SEQ ID NO:44)
AGA GGC CCA TTC ATC TAA CTC-3' (SEQ ID NO:45).

Page 47, beginning at line 13 through Page 48, line 3, replace these paragraphs with the following text:

-- Using the VAU sequence and its correlation with the MVP5180 and ANT70 sequences, oligonucleotide primers were defined which endeavor to be specific for the subgroup O in its entirety for the V3 region and the gp41 region. These primers made it possible to amplify the DUR strain and consequently constituted one solution to the amplification problem encountered. The position and the sequence of these HIV subgroup O primers are represented in Figure 13B and A respectively. These primers make it possible to obtain an amplification band which is visible on staining with ethidium bromide, with a single step of 30 cycles of PCR. Partial sequences were obtained:

- GAG: 513 base pairs (171 amino acids) = SEQ ID NO:95 and SEQ ID NO:96, Figure 10A and B
- gp120 V3 loop: 525 base pairs (75 amino acids) = SEQ ID NO:97 and SEQ ID NO:98, Figure 11A and B
- gp41 immunodominant region: 312 base pairs (104 amino acids) = SEQ ID N:99 and SEQ ID NO:100, Figure 12A and B.

Nucleotide (Figure 15A-C) and protein (Figure 16A-C) comparisons of the DUR sequences with the MVP5180, ANT and VAU sequences for the O subgroup, LAI for the HIV-1 consensus sequence, representative African HIV-1 MAL sequence, and CPZ for the CIV of the Gabonese chimpanzee, show that DUR is as remote from the other published HIV-1 group (or subgroup) O strains as the latter are from each other. --

Page 48, beginning at line 35 through page 49, line 2, replace this paragraph with the following text:

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-- In addition, it is possible to define in the GAG region segments common to the O group and to the M group, such as SPRTLNAWVK (SEQ ID NO:15), GSDIAGTTST (SEQ ID NO:16) and QGPKEPFRDYVDRF (SEQ ID NO:17). --

Page 49, beginning at line 9 through line 17, replace this paragraph with the following text:

-- The alignments of peptide sequences in the regions of the V3 loop of gp120 and in the immunodominant region of gp41 is given in Figure 9. The sequence of the interior of the V3 loop of the DUR strain differs substantially from that of the HIV-1 subgroup M consensus sequence. It shares the motif GPMAWYSM (SEQ ID NO:28) with the VAU and ANT70 strains but not with the MVP strain, which has two substitutions: R for A and R for Y. --

Page 50, beginning at line 14 through line 23, replace this paragraph with the following text:

-- The anti-DUR antiserum does not react with the peptides of the V3 loop of the HIV-1-M consensus sequence, of HIV-1 MAL, of HIV-1 CPZ or of HIV-1 group (or subgroup) O MVP5180 but does, however, react with the peptide of the V3 loop of HIV-1-O ANT70 as seen in Figure 14A. As regards the gp41 immunodominant region, this does not react with the "standard" HIV-1 subgroup M consensus sequence as seen in Figure 14B, but does, however, react, weakly but surprisingly, with the HIV-1 subgroup M right-extended consensus sequence. --

IN THE DRAWINGS:

Please amend the drawings as indicated below and in the attached Letter to the Office Draftsman, filed concurrently herewith.

In Figure 8C, please delete the last four amino acids letter designations "klae" in the line labeled "DUR" and insert therefor --lrae--.

In Figure 9A, please delete the "S" in the last six amino acids letter designations line labeled "VAU", which recite "S-A-Y-", and insert therefor --_--.

IN THE CLAIMS:

Please cancel claim 1 and add the following new claims:

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-- 30. (NEW) A method for producing an antibody against an HIV-1 type O virus comprising:

(a) inoculating an animal with an HIV-1 Env polypeptide comprising an amino acid sequence selected from the group consisting of:

CysValArgProGlyAsnAsnSerValLysGluIleLysIleGlyProMetAlaTrpTyrSerMetGlnIle GluArgGluGlyLysGlyAlaAsnSerArgThrAlaPheCys (SEQ ID NO:93);

CysLysAsnArgLeuIleCys (SEQ ID NO:5);

ArgLeuLeuAlaLeuGluThrPheIleGlnAsnTrpTrpLeuLeuAsnLeuTrpGlyCysLys AsnArgLeuIleCys (SEQ ID NO:6);

ArgAlaArgLeuLeuAlaLeuGluThrPheIleGlnAsnGlnGlnLeuLeuAsnLeuTrpGlyCys LysAsnArgLeuIleCysTyrThrSerValLysTrpAsnLysThr (SEQ ID NO:7);

LysGluIleLysIle (SEQ ID NO:23);

GluArgGluGlyLysGlyAlaAsn (SEQ ID NO:24);

CysValArgProGlyAsnAsnSerValLysGluIleLysIle (SEQ ID NO:25);

GlnIleGluArgGluGlyLysGlyAlaAsnSerArg (SEQ ID NO:26);

ArgLeuLeuAlaLeuGluThrLeuMetGlnAsnGlnGlnLeu (SEQ ID NO:29);

LeuAsnLeuTrpGlyCysArgGlyLysAlaIleCysTyrThrSerValGlnTrpAsnGluThrTrpGly (SEQ ID NO:30);

CysArgGlyLysAlaIle (SEQ ID NO:31);

SerValGlnTrpAsn (SEQ ID NO:32);

ArgLeuLeuAlaLeuGluThrLeuMetGlnAsnGlnGlnLeuLeuAsnLeuTrpGlyCysArgGly LysAlaIleCysTyrThrSer (SEQ ID NO:33);

GlnAsnGlnGlnLeuLeuAsnLeuTrpGlyCysArgGlyLysAlaIleCysTyrThrSerValGlnTrp Asn (SEQ ID NO:34);

ThrPheIleGlnAsn (SEQ ID NO:40);

TrpGlyCysLysAsnArg (SEQ ID NO:41) and

(b) isolating an antibody that binds to said HIV-1 Env polypeptide.

31. (NEW) The method of claim 30, wherein said method comprises isolating serum from said animal.

32. (NEW) A kit for the detection of antibodies against an HIV-1 type O virus in a biological sample comprising:

(a) a polypeptide comprising an amino acid sequence selected from the group consisting of:

CysValArgProGlyAsnAsnSerValLysGluIleLysIleGlyProMetAlaTrpTyrSerMetGlnIle GluArgGluGlyLysGlyAlaAsnSerArgThrAlaPheCys (SEQ ID NO:93);

CysLysAsnArgLeuIleCys (SEQ ID NO:5);

ArgLeuLeuAlaLeuGluThrPheIleGlnAsnTrpTrpLeuLeuAsnLeuTrpGlyCysLys AsnArgLeuIleCys (SEQ ID NO:6);

ArgAlaArgLeuLeuAlaLeuGluThrPheIleGlnAsnGlnGlnLeuLeuAsnLeuTrpGlyCys LysAsnArgLeuIleCysTyrThrSerValLysTrpAsnLysThr (SEQ ID NO:7);

LysGluIleLysIle (SEQ ID NO:23);

GluArgGluGlyLysGlyAlaAsn (SEQ ID NO:24);

CysValArgProGlyAsnAsnSerValLysGluIleLysIle (SEQ ID NO:25);

GlnIleGluArgGluGlyLysGlyAlaAsnSerArg (SEQ ID NO:26);

ArgLeuLeuAlaLeuGluThrLeuMetGlnAsnGlnGlnLeu (SEQ ID NO:29);

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LeuAsnLeuTrpGlyCysArgGlyLysAlaIleCysTyrThrSerValGlnTrpAsnGluThrTrpGly (SEQ ID NO:30);

CysArgGlyLysAlaIle (SEQ ID NO:31);

SerValGlnTrpAsn (SEQ ID NO:32);

ArgLeuLeuAlaLeuGluThrLeuMetGlnAsnGlnGlnLeuLeuAsnLeuTrpGlyCysArgGly LysAlaIleCysTyrThrSer (SEQ ID NO:33);

GlnAsnGlnGlnLeuLeuAsnLeuTrpGlyCysArgGlyLysAlaIleCysTyrThrSerValGlnTrp Asn (SEQ ID NO:34);

ThrPheIleGlnAsn (SEQ ID NO:40);

TrpGlyCysLysAsnArg (SEQ ID NO:41) and

(b) a detection reagent.

33. (NEW) The kit of claim 32, further comprising a negative control sample.

34. (NEW) The kit of claim 32, further comprising an incubation buffer.

35. (NEW) A method for preparing a lysate of an HIV-1 virus comprising:

(a) separating an HIV-1 virus from cells infected with said virus and

(b) lysing said virus,

wherein said virus comprises an Env protein that comprises an amino acid sequence selected from the group consisting of:

CysValArgProGlyAsnAsnSerValLysGluIleLysIleGlyProMetAlaTrpTyrSerMetGlnIle GluArgGluGlyLysGlyAlaAsnSerArgThrAlaPheCys (SEQ ID NO:93);

CysLysAsnArgLeuIleCys (SEQ ID NO:5);

ArgLeuLeuAlaLeuGluThrPheIleGlnAsnTrpTrpLeuLeuAsnLeuTrpGlyCysLys AsnArgLeuIleCys (SEQ ID NO:6);

ArgAlaArgLeuLeuAlaLeuGluThrPheIleGlnAsnGlnGlnLeuLeuAsnLeuTrpGlyCys LysAsnArgLeuIleCysTyrThrSerValLysTrpAsnLysThr (SEQ ID NO:7);

LysGluIleLysIle (SEQ ID NO:23);

GluArgGluGlyLysGlyAlaAsn (SEQ ID NO:24);

CysValArgProGlyAsnAsnSerValLysGluIleLysIle (SEQ ID NO:25);

GlnIleGluArgGluGlyLysGlyAlaAsnSerArg (SEQ ID NO:26);

ArgLeuLeuAlaLeuGluThrLeuMetGlnAsnGlnGlnLeu (SEQ ID NO:29);

LeuAsnLeuTrpGlyCysArgGlyLysAlaIleCysTyrThrSerValGlnTrpAsnGluThrTrpGly (SEQ ID NO:30);

CysArgGlyLysAlaIle (SEQ ID NO:31);

SerValGlnTrpAsn (SEQ ID NO:32);

ArgLeuLeuAlaLeuGluThrLeuMetGlnAsnGlnGlnLeuLeuAsnLeuTrpGlyCysArgGly LysAlaIleCysTyrThrSer (SEQ ID NO:33);

GlnAsnGlnGlnLeuLeuAsnLeuTrpGlyCysArgGlyLysAlaIleCysTyrThrSerValGlnTrp Asn (SEQ ID NO:34);

ThrPheIleGlnAsn (SEQ ID NO:40);

TrpGlyCysLysAsnArg (SEQ ID NO:41).

36. (NEW) The method of claim 35, further comprising pelleting said virus by centrifugation.

37. (NEW) The method of claim 35, further comprising isolating nucleic acid from said virus. --

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1300 I Street, NW
Washington, DC 20005
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REMARKS

No new matter enters through this amendment. Upon entry of the amendment, claims 30-37 are pending in this application.

The specification has been amended to include the section entitled THE BRIEF DESCRIPTION OF THE DRAWINGS to briefly describe that which is portrayed in each of the drawings of the application. Furthermore, the application has been amended to make particular reference to each of the Figures and sequence listings. The specification has further been amended to insert the address of the C.N.C.M. Depository.

The sequence of peptide (21) (SEQ ID NO:33) on pages 23 and 24 in the specification has been amended to correct an obvious typographical error. Applicants submit that the amendment to the specification does not introduce new matter. According to M.P.E.P. § 2163.07, an amendment to correct an obvious error does not constitute new matter where one skilled in the art would not only recognize the existence of the error in the specification, but also the appropriate correction. In re Oda, 443, F.2d 1200, 1206, 170 USPQ 268, (C.C.P.A. 1971).

Applicants submit that one skilled in the art recognizes the error in the specification on pages 23 and 24, in the recitation of "RLLALETLMONQQLLNLWGCRGKAICYTS", since "O" is not a one letter designation routinely used to designate amino acids. Applicants further submit that the skilled artisan recognizes the appropriate correction for this error, since a "Q" is found at this position in Figure 9B and 12 B, which show the immunodominant region of HIVDUR gp41; within the amino acid sequence "RLLALETLMQNQQL" (SEQ ID NO:29) on pages

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22 and 23 of the specification; and within the amino acid sequence "QNQQLLNLWGCRGKAICYTSVQWN" (SEQ ID NO:34) on pages 23 and 24 of the specification. Applicants submit that, because the skilled artisan would both recognize the error in the specification and the appropriate correction, no new matter is added to the specification by amendment.

Figure 8C has been amended to correct an obvious typographical error. Applicants submit that one skilled in the art recognizes the obvious error in Figure 8C, in the recitation of

". . . ktklae" at the end of the sequence alignments, since the amino acid sequence of Gag of HIVDUR is shown in Figure 10B (SEQ ID NO:96) to have a "KTLRAE" at this position. In addition, the skilled artisan recognizes that translation of the corresponding nucleotide sequence shown in Figure 10A, "AAAACATTAAGAGCTGAG", results in the sequence "KTLRAE", and not "ktklae". Furthermore, applicants submit that the skilled artisan recognizes the obvious error in the other sequences in Figure 8C, since, for example, the sequence of the corresponding amino acids in HIV-1MAL found in U.S. Patent No. 5,030,714 (Fig. 3A-1, a.a. 308-313) and HIV-1MVP1580 found in U.S. Patent No. 5,770,427 (Fig. 7, 1st 6 a.a. of the 7th line of alignments), are "KTLRAE". Therefore, applicants submit that, because the skilled artisan would both recognize the error in the specification and the appropriate correction, no new matter is added to the specification by amendment.

Figure 9A has been amended to correct an obvious typographical error. Applicants submit that one skilled in the art recognizes the obvious error in Figure 9A, in the recitation of "-E--G-QTIQK-MA__--_M-WYSMALSNTK-DT_S-A-Y-", since the amino

acid sequence of Env of HIVvau is shown in Figure 3B (SEQ ID NO:102) not to have an S (serine) at this position in the amino acid sequence, and the nucleotide sequence of Gag of HIVvau, shown in Figure 6, does not encode a serine at this position. Therefore, applicants submit that, because the skilled artisan would both recognize the error in the specification and the appropriate correction, no new matter is added to the specification by amendment.

Upon approval of the proposed changes to the Drawings, applicants respectfully request that the submission of revised formal drawings be deferred until after a Notice of Allowance has issued.

Claims 1-29 have been canceled. Claims 30-48 are new and find support throughout the specification, for instance, in original claims 1-29. The amendment has been made to conform the original claims to U.S. practice, and adds no new matter.

Applicants submit herewith a Sequence Listing and have amended the specification to conform with the requirements of 37 C.F.R. §§ 1.821-1.825.

Applicants hereby request that the computer-readable form of the sequence listing submitted in Serial No. 08/817,441 on August 31, 1998, be used in this application. I hereby state that the contents of the paper copy of the Sequence Listing submitted herewith and the computer-readable form of the Sequence Listing submitted in Serial No. 08/817,441 on August 31, 1998, are the same.

I further state that the submission, filed in accordance with 37 C.F.R. § 1.821(g) does not contain new matter. Applicants note that the Sequence Listing contains the same sequences as found within the specification as amended.

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DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

Please grant any extensions of time required to enter this response and charge any additional required fees our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: December 27, 2001

By: ______________________
Salvatore J. Arrigo
Reg. No. 46,063
Tel.: 202-408-4160 Fax: 202-408-4400
E-mail: arrigos@finnegan.com

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

CLAIMS

1. Nucleotide sequence, more particularly DNA and cloned DNA fragments which may be obtained from RNA, from cDNA or from primers which may be used for gene amplification, derived from the RNA or the DNA of the HIV-1 group O retrovirus, said nucleotide sequence being characterized in that it comprises the sequence corresponding to Seq ID No. 5, as well as any portion of this sequence or variant of this portion which is capable of hybridizing with the corresponding DNA or RNA of the HIV-1 group O virus.

2. Nucleotide sequence according to claim 1, characterized in that it is DNA or DNA fragments obtained from RNA, from cDNA or from primers for gene amplification, derived from the RNA or the DNA of the $HIV\text{-}1_{(VAU)}$ retrovirus, the sequence comprising the sequence corresponding to Seq ID No. 5 as well as any portion of this sequence or variant of this portion which is capable of hybridizing with the corresponding DNA or RNA of the $HIV\text{-}1_{(VAU)}$ virus.

3. Nucleotide sequence according to claim 1 or claim 2, characterized in that said sequence is chosen from the group of sequences corresponding to Seq ID No. 1, Seq ID No. 2, Seq ID No. 3 and Seq ID No. 4.

4. Nucleotide sequence, characterized in that it comprises the sequence of nucleotides corresponding to SEQ ID No. 7 and in that it codes for the integrase of an HIV-1 group O retrovirus, in particular of an $HIV\text{-}1_{(VAU)}$ retrovirus, or nucleotide sequence which hybridizes with the sequence containing the sequence SEQ ID No. 7.

5. Oligonucleotide comprising at least 9 nucleotides, as obtained from a nucleotide sequence according to any one of claims 1 to 4, which is capable of being

used as a primer for the gene amplification of an HIV-1 group O retrovirus.

6. Nucleotide sequence which may be used as a probe, characterized in that it hybridizes under highly stringent hybridization conditions with the DNA produced by gene amplification by means of primers according to claim 5.

7. Composition for the detection of the presence or absence of an HIV-1 group O retrovirus, in particular the HIV-$1_{(VAU)}$ retrovirus, in samples of serum or of other biological liquids or tissue obtained from patients suspected of being carriers of an HIV-1 group O retrovirus, said composition being characterized in that it comprises at least one probe obtained from a nucleotide sequence obtained from the genome of the HIV-$1_{(VAU)}$ virus, particularly an HIV-$1_{(VAU)}$ DNA fragment containing the *env* region or a part of the *env* region of the HIV-$1_{(VAU)}$ virus, of a variant of HIV-$1_{(VAU)}$ as defined in any one of claims 1 to 5.

8. Composition according to claim 7, characterized in that said composition also comprises a probe obtained from a nucleotide sequence obtained from HIV-1 not belonging to the O group, and/or from HIV-2.

9. Composition for the detection of the presence or absence of an HIV-1 group O retrovirus, in particular the HIV-$1_{(VAU)}$ retrovirus, in a biological sample, said composition being characterized in that it comprises at least two nucleotide sequences according to any one of claims 1 to 5, obtained from the genome of the HIV-$1_{(VAU)}$ virus, which sequences can be used as primers for amplification, in particular by PCR, of the DNA and/or RNA of HIV-1 group O retrovirus and in particular of HIV-$1_{(VAU)}$.

10. Nucleotide sequence, characterized in that it is an RNA sequence corresponding to a DNA sequence according to any one of claims 1 to 7.

11. Envelope protein of the HIV-$1_{(VAU)}$ retrovirus, characterized in that it may be obtained by expression, in a host cell, of a nucleotide sequence according to claim 1, and in that said protein comprises the amino acid sequence between residues 1 and 526 of Seq ID No. 6, as well as any peptide, polypeptide, glycoprotein or variant derived from said sequence having an epitope which is capable of being recognized by antibodies induced by the HIV-$1_{(VAU)}$ virus.

12. Envelope protein of the HIV-$1_{(VAU)}$ retrovirus, characterized in that it may be obtained by expression, in a host cell, of a nucleotide sequence according to claim 1, and in that said protein comprises the amino acid sequence between residues 527 to 877 of Seq ID No. 7, as well as any peptide, polypeptide, glycoprotein or variant derived from said sequence having an epitope which is capable of being recognized by antibodies induced by the HIV-$1_{(VAU)}$ virus.

13. Peptide or polypeptide according to claim 11 or 12, characterized in that it comprises the sequence CKNRLIC or in particular the sequence RLLALETFIQNWWLLNLWGCKNRLIC or a variant of that sequence such as the sequence RLWALETLIQNQQRLNLWGCKGKLIC, the sequence RLLALETLLQNQQLLSLWGCKGKLVC or the sequence RARLLALETFIQNQQLLNLWGCKNRLICYTSVKWNKT.

14. Synthetic peptide, characterized in that it is a protein fragment according to either of claims 11 and 12, in that it is obtained from the sequence SEQ ID No. 6 or from the sequence SEQ ID No. 7 and in that it is recognized by antibodies induced against an HIV-$1_{(VAU)}$ retrovirus or variant of this fragment capable of being recognized by antibodies induced by an HIV-$1_{(VAU)}$ retrovirus.

15. Composition for the in vitro detection of the presence, in a human biological sample, of anti-HIV-$1_{(VAU)}$ antibodies, said composition comprising at least one antigen comprising a protein, a glycoprotein, a polypeptide or a peptide of the envelope protein of an HIV-$1_{(VAU)}$ retrovirus as defined in any one of claims 11 to 14.

16. Composition according to claim 15, characterized in that it also comprises an antigen such as a protein, a glycoprotein, a polypeptide or a peptide of an HIV-1 virus not belonging to the O group and/or of an HIV-2 virus or a peptide derived from an HIV-1 virus not belonging to the O group and/or from an HIV-2 virus having an epitope which may be recognized by the antibodies induced by the HIV-1 virus not belonging to the O group and/or the HIV-2 virus.

17. Composition according to claim 16, characterized in that the proteins and/or glycoproteins of HIV-1 not belonging to the O group and/or of HIV-2 are gag or pol proteins or peptides thereof.

18. Composition according to claim 16, characterized in that the proteins and/or glycoproteins of HIV-1 not belonging to the O group and/or of HIV-2 are envelope glycoproteins.

19. Composition according to any one of claims 15 to 18, characterized in that said composition comprises a peptide sequence corresponding to the entire region 590-620 of the gp41 protein of HIV-$1_{(VAU)}$ or a part of this region which is specific for HIV-$1_{(VAU)}$.

20. Composition according to claim 19, characterized in that said peptide sequence is the sequence -TFIQN-, CKNRLIC or WGCKNR.

21. Antibody which may recognize an envelope protein, a peptide or a polypeptide derived from said envelope protein according to claim 11.

22. Process for the in vitro diagnosis of an infection caused by the HIV-$1_{(VAU)}$ virus, said process comprising:

- the placing in contact of a serum or of another biological medium, obtained from a patient forming the subject of the diagnosis, with at least one of the envelope proteins or glycoproteins of the HIV-$1_{(VAU)}$ virus or of a peptide or polypeptide obtained from one of these proteins or glycoproteins according to any one of claims 11 to 14 or a composition according to any one of claims 15 to 20, and

- the detection of an immunological reaction.

23. Reagent required for the Western blot (immunoblot) or ELISA reaction, containing an envelope protein or glycoprotein of the HIV-$1_{(VAU)}$ virus or of a peptide or polypeptide obtained from one of these proteins or glycoproteins according to any one of claims 11 to 14 or a composition according to any one of claims 15 to 20.

24. Use of a nucleotide sequence according to claim 1 or 2 in order to induce in vivo the synthesis of antibodies directed against the antigen coded for by said sequence.

25. Immunogenic composition according to any one of claims 15 to 20, which is capable of inducing antibodies in animals.

26. Diagnostic kit for the <u>in vitro</u> detection, on a biological sample, of an infection with an HIV-1 group O retrovirus, for example of an HIV-$1_{(VAU)}$ retrovirus, characterized in that it comprises:

- primers according to claim 5 for the gene amplification of an HIV-1 group O retrovirus,

- reagents required for the gene amplification reaction.

27. Kit for the <u>in vitro</u> detection, on a biological sample, of an HIV-1 group O retrovirus, characterized in that it comprises as optionally labeled probe, at least one nucleotide sequence according to one of claims 1, 2, 3, 4, 5, 6 or 10 or a composition according to one of claims 7, 8 or 9, and optionally another nucleotide probe according to any one of claims 1 to 6 or composition according to any one of claims 7, 8 or 9, which is optionally immobilized on a solid support.

28. Kit according to claim 27, characterized in that it also comprises the reagents required for carrying out a hybridization.

29. Bacterial strain deposited at the CNCM on 20 October 1994 under the access number I-1486.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

Page 8, beginning at line 26 through page 9, line 2, this paragraph has been amended as follows:

Two weeks after coculturing the patient's CD8-depleted, PHA-stimulated PBMCs with similar cells from a healthy donor, the production of virus was detected in the form of an RT activity peak in the culture supernatant. This virus could then be subjected to serial passages on CD8-depleted, PHA-stimulated normal PBMCs. Figure ~~1, plate A~~ 1A represents the production of HIV-$1_{(VAU)}$ in infected PBMC culture supernatants, checked by RT assay (filled circles) and HIV-1 p24 antigen capture ELISA (empty circles). The concentration of HIV-1 p24 is expressed in ng/ml and the RT activity in cpm/μl. In ~~plate B~~ Figure 1B, the same experiment was carried out with a standard primary HIV-1 isolate from an AIDS patient.

Page 11, beginning at line 14 through line 20, this paragraph has been amended as follows:

The invention relates to any variant of the nucleic acid sequences of the HIV-$1_{(VAU)}$ virus or of any group O equivalent virus, containing structural proteins which have the same immunological properties as the structural proteins coded for by the env gene comprising the sequence described in Figure 6 and called "vau", also designated by SEQ ID ~~No. 5~~ NO:63.

Page 11, beginning at line 33 through page 12, line 11, this paragraph has been amended as follows:

The invention relates to the DNAs or DNA fragments, more particularly cloned DNAs and DNA fragments, obtained from RNA, cDNA or primers which can be used in PCR, or other gene amplification methods, derived from the HIV-$1_{(VAU)}$ retrovirus RNA or DNA. The invention relates more particularly to all the equivalent DNAs, especially to any DNA having sequence homologies with the HIV-$1_{(VAU)}$ DNA, in particular with the sequence coding for ~~for~~ the *env* region of the $HIV_{(VAU)}$ strain comprising the sequence corresponding to SEQ ID ~~No. 5~~ NO:63 represented in Figure 6 and called "vau". The homology with HIV-1 group M is at least equal to 50%, preferably to 70% and still more advantageously to about 90%. Generally, the invention relates to any equivalent DNA (or RNA) capable of hybridizing with the DNA or RNA of a group O HIV-1 retrovirus.

Page 12, beginning at line 14 through line 18, this paragraph has been amended as follows:

--The invention also relates to the HIV-$1_{(VAU)}$ integrase gene comprising the sequence identified by the same SEQ ID ~~No. 7~~ NO:64 or hybridizing with SEQ ID ~~No. 7~~ NO:64. The invention also relates to the RNAs corresponding to the DNA described above.

Page 13, beginning at line 19 through page 14, line 11, this paragraph has been amended as follows:

The resulting amplification product was cloned into a pBluescript vector, generating the clone ph4, deposited at the ~~CNCM~~ Collection Nationale des Cultures de Micro-organismes (CNCM), Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15 France, on 20 October 1994 under No. I-1486, which was subsequently used as a probe to screen a lambda library of low molecular weight DNA, which was digested with EcoRI and was obtained from cells infected with HIV-$1_{(VAU)}$. Briefly, the PBMCs infected with HIV-$1_{(VAU)}$ were cocultured for 24 hours with new PBMCs stimulated with PHA and depleted of $CD8^+$ cells, after which a high cytopathic effect (CPE) was visible. The low molecular weight DNA was then extracted according to the Hirt method (Hirt 1967), and digested with the enzyme EcoRI. A previous Southern-blot analysis of this DNA had indeed shown that the HIV-$1_{(VAU)}$ genome contained only one EcoRI site, permitting the cloning of nonintegrated circular DNA species representing the entire viral genome. The resulting digestion product was subjected to agarose gel electrophoresis, and the population of DNA fragments of approximately 8-12 kb in size was purified and ligated to EcoRI-digested lambda Zap DNA (Stratagene). After encapsidation, plating and screening by hybridization with ^{32}P-labeled ph4 DNA, a clone, λH34, was identified as being positive, and amplified. The EcoRI insert was purified, sonicated, and cloned by the "shotgun" technique into the phosphatase-treated vector M13mp18 digested with the enzyme SmaI. One hundred and fifty of the clones obtained were sequenced in a 373A DNA sequencer (Applied Biosystems), and the resulting sequences were assembled into a single sequence using the Wisconsin GCG DNA analysis package.

Page 14, beginning at line 24 through line 36, this paragraph has been amended as follows:

The PCR amplification was carried out in 35 thermal cycles at 92°C for 15 seconds, 52°C for 1 minute, 60°C for 2 minutes and 72°C for 2 minutes. The resulting amplification product, of 3.5 kb in size, was cloned into the M13mp18 vector and sequenced by successive reactions, first using the M13 universal sequencing primer, and then the primers deduced from the upstream sequences. Analysis of the nucleotide and peptide sequences was carried out using the Wisconsin GCG DNA analysis package. The HIV-$1_{(VAU)}$ *env* gene codes for 877 amino acids in total, including the signal peptide. The nucleotide sequence of the HIV-$1_{(VAU)}$ *env* gene corresponds to SEQ ID ~~No. 5~~ NO:63 (see Figure 3).

Page 15, beginning at line 29 through line 37, this paragraph has been amended as follows:

Particularly advantageous are the probes which, when hybridized with HIV-1, give a strong reaction with HIVs belonging to group O and weak reaction with HIVs belonging to group M. By way of nonlimiting example, a probe constructed from the HIV-$1_{(VAU)}$ virus integrase gene sequence SEQ ID ~~No. 7~~ NO:64 gives, when it is hybridized with HIV-1 under hybridization conditions such as those described in Patent EP 178 978, a strong reaction with group O HIVs and a weak reaction with group M HIVs.

Page 17, beginning at line 13 through line 23, this paragraph has been amended as follows:

The invention relates to an external envelope protein of the HIV-$1_{(VAU)}$ retrovirus encoded by the gene comprising the sequence corresponding to SEQ ID ~~No. 5~~ NO:63. According to a preferred embodiment of the invention, this protein is in addition characterized in that it comprises the amino acid sequence corresponding to SEQ ID ~~No. 6~~ NO:46 represented in Figure 3 and comprising amino acid residues 1 to 526. The subject of the invention is also any polypeptide or variant which is derived from said sequence having an epitope which may be recognized by the antibodies induced by the HIV-$1_{(VAU)}$ virus.

Page 17, beginning at line 26 through line 31, this paragraph has been amended as follows:

The subject of the invention is also an envelope transmembrane protein comprising the amino acid sequence SEQ ID ~~No. 8~~ NO:47 represented in Figure 3 between amino acid residues 527 and 877. This transmembrane protein is, within the scope of the invention, in glycosylated or nonglycoslated form.

Page 19, beginning at line 7 through line 17, these paragraphs have been amended as follows:

Preferred polypeptides of this region are, for example, those which contain the sequence CKNRLIC (SEQ ID NO:5) or correspond to this sequence. They may also be peptides or polypeptides corresponding to the sequence RLLALETFIQNWWLLNLWGCKNRLIC (SEQ ID NO:6) or comprising this sequence.

Another preferred peptide, identified below by the name "VAU peptide", corresponds to the following sequence or comprises this sequence or any part of this

sequence capable of being recognized by antibodies directed against the HIV-$1_{(VAU)}$ retrovirus RARLLALETFIQNQQLLNLWGCKNRLICYTSVKWNKT (SEQ ID NO:7).

Page 19, beginning at line 24 through line 31, this paragraph has been amended as follows:

The present invention relates to a peptide obtained from the HIV-1-O DUR virus deposited on 23 February 1995 at the ~~CNCM~~ Collection Nationale des Cultures de Micro-organismes (CNCM), Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15 France, under the reference I-1542, or a peptide whose sequence is distinguished from that of the above by substitution, deletion or addition of amino acids, this separate peptide nevertheless retaining the antigenic characteristics of the above one.

Page 19, beginning at line 34 through page 21, line 9, these paragraphs have been amended as follows:

Thus, a preferred peptide of the invention is a peptide containing at least 4 consecutive amino acids contained in the GAG sequence represented in Figure 8A-C or in an immunologically similar GAG sequence obtained from a variant of the HIV-1-O DUR virus, said immunologically similar sequence being recognized by antibodies which also specifically recognize at least one of the sequences AHPQQA (SEQ ID NO:8), LWTTRAGNP (SEQ ID NO:9) contained in the GAG sequence of Figure 8.

Preferably, this peptide consists of a peptide whose amino acid sequence is contained either in one of the following sequences:

SPRTLNAWVKAVEEKAFNPEIIPMFMALSEGA (1) (SEQ ID NO:10)
MLNAIGGHQGALQVLKEVIN (2) (SEQ ID NO:11)
GPLPPGQIREPTGSDIAGTTSTQQEQI (3) (SEQ ID NO:12)
IPVGDIYRKWIVLGLNKMVKMYSPVSILDI (4) (SEQ ID NO:13)
QGPKEPFRDYVDRFYKTKLAE (5) (SEQ ID NO:14)
AHPQQA (5a) (SEQ ID NO:8)
LWTTRAGNP (5b) (SEQ ID NO:9)

or in a corresponding immunologically similar sequence, this peptide containing at least four consecutive amino acids of one of said sequences.

Preferably also, this peptide consists of a peptide whose amino acid sequence is contained either in one of the following sequences:

SPRTLNAWVK (6) (SEQ ID NO:15)
GSDIAGTTST (7) (SEQ ID NO: 16)
QGPKEPFRDYVDRF (8) (SEQ ID NO: 17)

or in a corresponding immunologically similar sequence, this peptide containing at least four consecutive amino acids of one of said sequences.

Peptides which are particularly preferred in the present invention are the peptides containing:

-the amino acid sequence NPEI (9) (SEQ ID NO:18)

or

- the amino acid sequence AVEEKAFNPEIIPMFM (10) (SEQ ID NO:19), and more particularly peptides whose amino acid sequence is contained, either in one of the following sequences:

IGGHQGALQ (23) (SEQ ID NO:20)

REPTGSDI (24) (SEQ ID NO:21)

or in a corresponding immunologically similar sequence, this peptide containing at least 4 consecutive amino acids of one of said sequences, as well as the peptide whose amino acid sequence is contained, in the following amino acid sequence:

IDEAADWD (25) (SEQ ID NO:22)

or in a corresponding immunologically similar sequence, this peptide containing at least four consecutive amino acids of said sequence.

Page 21, beginning at line 20 through line 33, this paragraph has been amended as follows:

A peptide derived from the HIV-1-O DUR virus defined above also falls within the scope of the present invention, said peptide containing at least 4 consecutive amino acids of the V3 loop of gp120 represented in Figure 9A or of the corresponding immunologically similar sequence, obtained from a variant of the HIV-1-O DUR virus, said immunologically similar sequence being recognized by antibodies which also specifically recognize at least one of the sequences:

KEIKI (12) (SEQ ID NO:23)

EREGKGAN (13) (SEQ ID NO: 24)

CVRPGNNSVKEIKI (14) (SEQ ID NO:25)

QIEREGKGANSR (15) (SEQ ID NO:26).

Page 21, beginning at line 35 through page 22, line 14, this paragraph has been amended as follows:

This peptide preferably contains:

a) either the sequence CVRPGNNSVKEIKIGPMAWYSMQIEREGKGANSRTAFC (11) (SEQ ID NO: 27) or a part of this sequence which contains at least 4 amino acids

b) or an amino acid sequence which is separate from the sequence of a) in which one or more amino acids are replaced with one or more amino acids, with the proviso that the peptide retains its reactivity with an antiserum against the abovementioned peptide,

c) or an amino acid sequence which is separate from a) or b), in which one or more amino acids have been deleted or added, with the proviso that the peptide retains its reactivity with an antiserum against the peptide of a),

d) or a corresponding immunologically similar sequence or part of a sequence.

Page 22, beginning at line 16 through page 23, line 5, these paragraphs have been amended as follows:

Preferably also, this peptide contains either
the sequence KEIKI (12) (SEQ ID NO:23),
or
the sequence EREGKGAN (13) (SEQ ID NO:24),
or
the sequence GPMAWYSM (16) (SEQ ID NO:28).

In a particularly preferred manner, a peptide as defined above contains the amino acid sequence CVRPGNNSVKEIKI (14) (SEQ ID NO:25) or the sequence QIEREGKGANSR (15) (SEQ ID NO:26).

A peptide derived from the HIV-1-O DUR virus as defined above also falls within the scope of the invention, said peptide containing at least 4 consecutive amino acids, whose entire sequence is contained in the sequence of the immunodominant region of gp41 represented in Figure 9B or in a corresponding immunologically similar sequence, obtained from a variant of the HIV-1-O DUR virus, said immunologically similar sequence being recognized by antibodies which also specifically recognize at least one of the following sequences:

RLLALETLMQNQQL (17) (SEQ ID NO:29),
LNLWGCRGKAICYTSVQWNETWG (18) (SEQ ID NO:30),
CRGKAI (19) (SEQ ID NO:31),
SVQWN (20) (SEQ ID NO:32),
~~RLLALETLMQNQQLLNLWGCRGKAICYTS~~
RLLALETLMQNQQLLNLWGCRGKAICYTS (21) (SEQ ID NO:33),
QNQQLLNLWGCRGKAICYTSVQWN (22) (SEQ ID NO:34).

Page 23, beginning at line 7 though line 27, this paragraph has been amended as follows:

This peptide is preferably a peptide containing the sequence RLLALETLMQNQQL (17) (SEQ ID NO:29) or LNLWGCRGKAICYTSVQWNETWG (18) (SEQ ID NO:30) or part of this peptide (18) (SEQ ID NO:30) containing:

a) either the sequence CRGKAI (19) (SEQ ID NO:31) or the sequence SVQWN (20) (SEQ ID NO:32) in which Q is, where appropriate, replaced by a different amino acid, which is nevertheless also different from K, or the two sequences at the same time,

b) or an amino acid sequence which is separate from the sequence of a) in which one or more amino acids are replaced with two amino acids, with the proviso that the peptide retains its reactivity with an antiserum against the peptide of a),

c) or an amino acid sequence which is separate from a) or b), in which one or more amino acids have been deleted or added, with the proviso that the peptide retains its reactivity with an antiserum against the peptide of a),

d) or in a corresponding immunologically similar sequence or part of a sequence.

Page 23, beginning at line 29 through page 24, line 4, this paragraph has been amended as follows:

Preferably also, this peptide possesses one or the other of the following characteristics:

- its N-terminal sequence which contains at least 8 amino acids is not immunologically recognized by antibodies formed against the sequence RILAVERY (SEQ ID NO:35) contained in the immunodominant region of gp41 of the HIV-1-LAI strain.
- it is not recognized by antibodies formed against the peptide SGKLIC (SEQ ID NO:36) of the HIV-1-LAI strain.
- it contains either of the following two sequences:

~~RLLALETLMQNQQLLNLWGCRGKAICYTS~~
RLLALETLMQNQQLLNLWGCRGKAICYTS (21) (SEQ ID NO:33)
QNQQLLNLWGCRGKAICYTSVQWN (22) (SEQ ID NO: 34).

Page 25, beginning at line 9 through line 25, this paragraph has been amended as follows:

The peptide is checked by HPLC and by mass spectrometry according to the electrospray technique (Figures 18A-B and Figures 19A-B) (FISON VG Trio 2000 spectrophotometer).

Fmoc: 9-Fluoroenylmethyloxycarbonyl
Pmc: 8-Methylpentane-6-sulfonylchroman
Trt: Tritryl
Boc: Tertbutyloxycarbonyl
tBU: tert butyl
DMF: Dimethylformamide
DIPCDI: Diisopropylcarbodiimide
HOBT: 1-Hydorxybenzotriazole
TF: Trifluoroacetic acid

Reagent K: Phenol/water/thioanisole/ethanedithiol/TFA: 2.5 ml/2.5 ml/2.5 ml/1.5 ml/41 ml

Comparison of the amino acid sequence of the HIV-$1_{(VAU)}$ envelope with the corresponding sequence of other HIV viruses.

Page 26, beginning at line 31 through page 27, line 9, this paragraph has been amended as follows:

The alignment of Figure 3 shows numerous regions of high divergence, with a few domains retained here and there. These retained regions correspond roughly to the domains also retained in the conventional HIV-1 isolates (Alizon et al. 1986, Benn et al. 1985). Among the divergent domains, the V3 loop, also called principal determinant of neutralization (Javaherian et al. 1990, Javaherian et al. 1989, Matsushita et al. 1988) is clearly one of the most divergent, although the two cysteines defining the loop are retained. The sequence of the cap of the loop, GPGRAF (SEQ ID NO:37) for HIV-1-LAI is GPMAWY (SEQ ID NO:38) in HIV-$1_{(VAU)}$. This unit of the cap is identical to that of the Cameroonian group O isolate $HIV_{(ANT70)}$ (Van den Heasevelde et al. 1994), but is different from that of the other group O isolate, $HIV_{(MVP5180)}$ (Gűrtler et al. 1994), for which the motif is GPMRWR (SEQ ID NO:39).

Page 30, beginning at line 30 through page 31, line 16, this paragraph has been amended as follows:

Generally, the invention relates to any composition which can be used for the in vitro detection of the presence, in a biological fluid, especially from individuals who have been brought into contact with HIV-$1_{(VAU)}$, or with antibodies against at least one of the HIV-$1_{(VAU)}$ antigens. This composition can be applied to the selective diagnosis of infection by an HIV-1 group O by using diagnostic techniques such as those described in Patent Applications EP 84401834 and EP 87400,151,4. Within the context of the present invention, any constituent comprising antigenic determinants capable of being recognized by antibodies produced against HIV-$1_{(VAU)}$ is used, for example recombinant antigens or peptides or chemically synthesized peptides defined from the sequence of the HIV-$1_{(VAU)}$ envelope. In this regard, the invention relates more particularly to compositions containing at least one of the HIV-$1_{(VAU)}$ virus envelope proteins. There may be mentioned, by way of examples of compositions, those which contain proteins, glycoproteins or peptides from the envelope protein corresponding to the entire 590-620 region of the HIV-$1_{(VAU)}$ gp41 protein or to the parts of this region which are specific for HIV-$1_{(VAU)}$ such as the peptides -TFIQN- (SEQ ID NO:40) or -WGCKNR- (SEQ ID NO:41).

Page 37, beginning at line 21, through page 38, line 13, this paragraph has been amended as follows:

The experimental data collated in the two tables of Figures ~~21~~ 20A-C and ~~22~~ 21A-C show that:

a) the four sera taken from patients contaminated with the HIV-1 group (or subgroup) O virus are very reactive with the vau peptide;

b) the ten sera supposedly taken from patients contaminated with the HIV-1 group (or subgroup) O virus, among the 19 sera sent out by the Pasteur Institute of Yacoundé, are also highly reactive with this same peptide;

c) the sera (4 samples) taken from individuals contaminated with the HIV-1 subtype B virus (in the acute phase) are not reactive with the vau peptide;

d) the sera taken from asymptomatic blood donors (48 samples tested) are not reactive with the vau peptide; These experimental data, although limited (in view of the ~~paupicity~~ paucity of HIV-1 group (or subgroup O) antibody-positive samples), bear witness to the sensitivity and specificity of the peptide selected.

Page 40, beginning at line 10 through line 16, this paragraph has been amended as follows:

According to the present invention, the process of detection and discrimination between infection by an HIV-1 group (or subgroup) O retrovirus and an HIV-1 subgroup M retrovirus is characterized by placing serum, obtained from individuals subjected to an AIDS diagnostic test, in contact, in particular, with the peptide RILAVERY (SEQ ID NO:35).

Page 45, beginning at line 19 through line 26, this paragraph has been amended as follows:

Oligonucleotide primers also according to the invention have a sequence consisting of at least eight consecutive nucleotides of the following nucleotide sequences:

ATT CCA ATA CAC TAT TGT GCT CCA-3' (SEQ ID NO:42)
AAA GAA TTC TCC ATG ACT GTT AAA-3' (SEQ ID NO:43)
GGT ATA GTG CAA CAG CAG GAC AAC-3' (SEQ ID NO:44)
AGA GGC CCA TTC ATC TAA CTC-3' (SEQ ID NO:45).

Page 47, beginning at line 13 through Page 48, line 3, these paragraphs have been amended as follows:

Using the VAU sequence and its correlation with the MVP5180 and ANT70 sequences, oligonucleotide primers were defined which endeavor to be specific for the subgroup O in its entirety for the V3 region and the gp41 region. These primers made it possible to amplify the DUR strain and consequently constituted one solution to the amplification problem encountered. The position and the sequence of these HIV subgroup O primers are represented in Figure 13B and A respectively. These primers make it possible to obtain an amplification band which is visible on staining with ethidium bromide, with a single step of 30 cycles of PCR. Partial sequences were obtained:

- GAG: 513 base pairs (171 amino acids) = ~~Seq ID No. 9~~ SEQ ID NO:95 and SEQ ID NO:96, Figure 10A and B
- gp120 V3 loop: 525 base pairs (75 amino acids) = ~~Seq ID No. 10~~ SEQ ID NO:97 and SEQ ID NO:98, Figure 11A and B
- gp41 immunodominant region: 312 base pairs (104 amino acids) = ~~Seq ID No. 11~~ SEQ ID N:99 and SEQ ID NO:100, Figure 12A and B.

Nucleotide (Figure 15A-C) and protein (Figure 16A-C) comparisons of the DUR sequences with the MVP5180, ANT and VAU sequences for the O subgroup, LAI for the HIV-1 consensus sequence, representative African HIV-1 MAL sequence, and CPZ for the CIV of the Gabonese chimpanzee, show that DUR is as remote from the other published HIV-1 group (or subgroup) O strains as the latter are from each other.

Page 48, beginning at line 35 through page 49, line 2, this paragraph has been amended as follows:

In addition, it is possible to define in the GAG region segments common to the O group and to the M group, such as SPRTLNAWVK (SEQ ID NO:15), GSDIAGTTST (SEQ ID NO:16) and QGPKEPFRDYVDRF (SEQ ID NO:17).

Page 49, beginning at line 9 through line 17, this paragraph has been amended as follows:

The alignments of peptide sequences in the regions of the V3 loop of gp120 and in the immunodominant region of gp41 is given in Figure 9. The sequence of the interior of the V3 loop of the DUR strain differs substantially from that of the HIV-1 subgroup M consensus sequence. It shares the motif GPMAWYSM (SEQ ID NO:28) with the VAU and ANT70 strains but not with the MVP strain, which has two substitutions: R for A and R for Y.

Page 50, beginning at line 14 through line 23, this paragraph has been amended as follows:

The anti-DUR antiserum does not react with the peptides of the V3 loop of the HIV-1-M consensus sequence, of HIV-1 MAL, of HIV-1 CPZ or of HIV-1 group (or subgroup) O MVP5180 but does, however, react with the peptide of the V3 loop of HIV-1-O ANT70 as seen in Figure 14A. As regards the gp41 immunodominant region, this does not react with the "standard" HIV-1 subgroup M consensus sequence as seen in Figure 14B, but does, however, react, weakly but surprisingly, with the HIV-1 subgroup M right-extended consensus sequence.

PATENT
Customer No. 22,852
Attorney Docket No. 02356.0076-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Pierre CHARNEAU et al.	)	
	)	Prior Group Art Unit: 1648
Serial No.: Not assigned	)	
(Based on Serial No.: 08/817,441)	)	Prior Examiner: R. Budens
	)	
Filed: December 27, 2001	)	

For: NUCLEOTIDE SEQUENCES OF HIV-1 TYPE (OR SUBTYPE) O RETROVIRUS ANTIGENS

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

REQUEST FOR APPROVAL OF DRAWING CHANGE

Subject to the approval of the Examiner, it is respectfully requested that Figs. 8C and 9A in the above-captioned application be amended as follows:

In Figure 8C, please delete the last four amino acids letter designations "klae" in the line labeled "DUR" and insert therefor --lrae--.

In Figure 9A, please delete the "S" in the last six amino acids letter designations line labeled "VAU", which recite "S-A-Y-", and insert therefor --_--.

The requested changes are indicated in red on the attached copies of the originally filed drawings. These changes provide corrections for obvious errors in the drawings of the specification without the introduction of new matter.

Formal drawings containing the requested changes are filed herewith.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

If any fees are necessary for the submission of these formal drawings, please charge our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: December 27, 2001

By: _____

Salvatore J. Arrigo
Reg. No. 46,063
Tel.: 202-408-4160
Fax: 202-408-4400
E-mail: arrigos@finnegan.com

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

```
                  # #     #
DUR  vgdiyrkwivlglnkmvkmyspvsildirqgpkepfrdyvdrfykt[klae]---lrae--
ANT  ----------------------k-----------------------------------
MVP  ----------------------------------------------------------
LAI  --e--kr--i-----i-r-----t----------------------f-----------
MAL  -----kr--i-----i-r----------------------------f-----------
CPZ  ---v--r-vi----v-r--g----------------------------------------

HIV1-M / HIV1-O discriminating positions  : #

Hypervariable regions  :  III
```

FIGURE 8C

REGION OF THE V3 LOOP OF GP120

```
----YK---QRTG-__---Q-LY-__THR-I-DI__-----          MAD
CTRPNNNTRKSIRIQRGPGRAFVT__IGK_IGNM__RQAHC          LAI
----------NR-S-_------H--__TKQ-I-DI__-----         OYI
-A--YQ---QRTP-__-L-QSLY-__TR_SRSII__G----          ELI
----G----RGIHF__---QALY-__T-_-V-DI__-R-Y-          MAL
-S--Y-TRKNIRRYSI-S-QAFYV__T---I-DI__-Q---          455
-H--G----GE__VQI---MTFYN__-ENVV-DT__-S-Y-          CPZ

-E--QI_DIQE__MRI--_M-WYSMG--GTA--S_S---Y-          ANT
-I-EGIAEVQD-YT__--_MRWRSM_TLIRSNNT_S-V-Y-          MVP
-E--G-QTIQK-MA__--_M-WYSMALSNTK-DT_A-Y-            VAU
-V--G--SV-E-K-__--_M-WYSMQ-EREGKGANS-T-F-          DUR

C*R*********I****GP_M*W*SM**********SR*A*C    HIV-O Consensus

CVRPGNNSVKEIKI__GP_MAWYSMQIEREGKGANSRTAFC          DUR
```

FIGURE 9A

CORRECTED SHEET (RULE 91)
ISA/EP

PATENT
Customer No. 22,852
Attorney Docket No. 2356.0076-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Pierre CHARNEAU et al.	)	Group Art Unit: 1648 (Prior Appln.)
	)	
Serial No.: To Be Assigned (Based on USSN: 08/817,441)	)	Examiner: R. Budens (Prior Appln.)
	)	
Filed: December 27, 2001	)	
	)	

For: NUCLEOTIDE SEQUENCES OF HIV-1 TYPE (OR SUBTYPE) O RETROVIRUS ANTIGENS

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

SUBMISSION OF FORMAL DRAWINGS

Subject to the approval of the Examiner, applicants submit herewith 37 sheets of formal drawings (Figures 1A, 1B, 2, 3A, 3B, 3C, 3D, 4, 5A, 5B, 6, 7, 8A, 8B, 8C, 9A, 9B, 10, 11, 12, 13A, 3B, 14, 15A, 15B, 16A, 16B, 17, 18A, 18B, 19, 20, 21A, 21B, 22A, 22B, and 22C). If the formal drawings for any reason are not in full compliance with the pertinent statutes and regulations, please so advise the undersigned.

If any fees are necessary for the submission of these formal drawings, please charge our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: December 27, 2001

By: _____
Salvatore J. Arrigo
Reg. No. 46,063
Tel.: 202-408-4160
Fax: 202-408-4400
E-mail: arrigos@finnegan.com

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

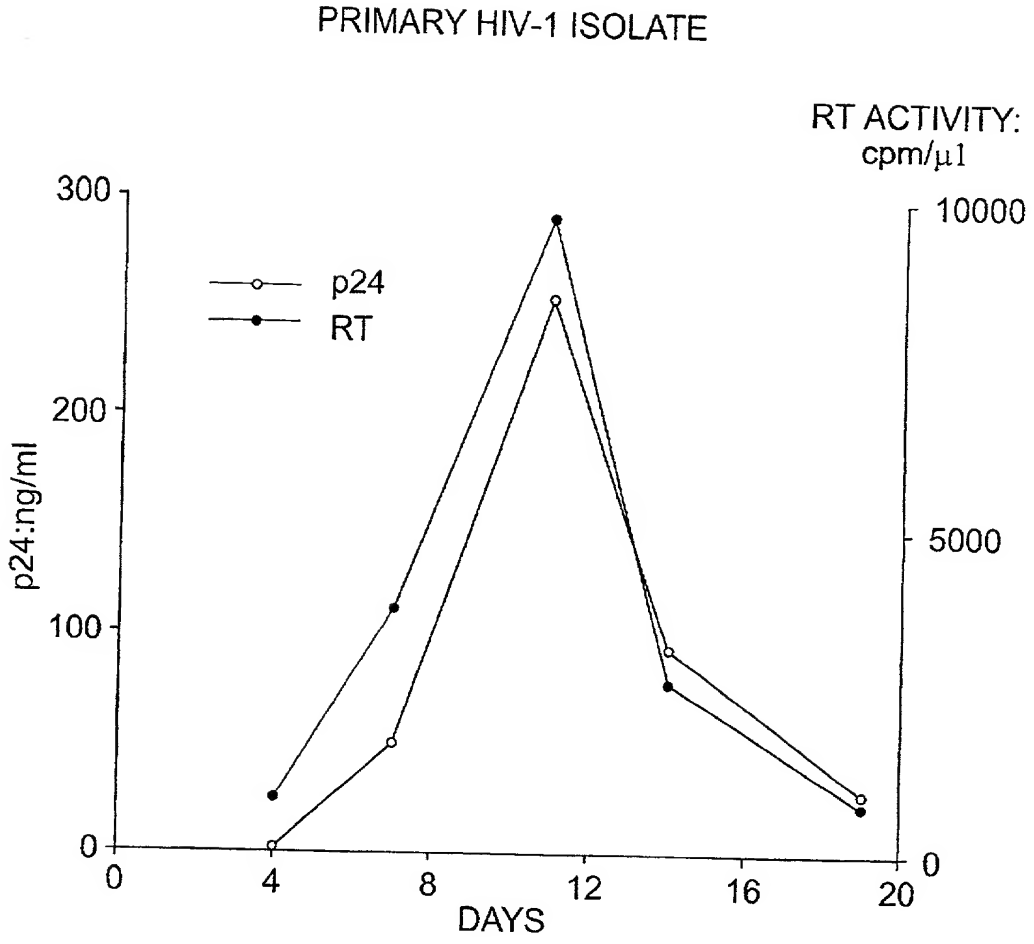
PRIMARY HIV-1 ISOLATE
RT ACTIVITY:
cpm/μl
10000
5000
0
300
200
100
0
p24:ng/ml
p24
RT
0
4
8
12
16
20
DAYS

FIG. 1A

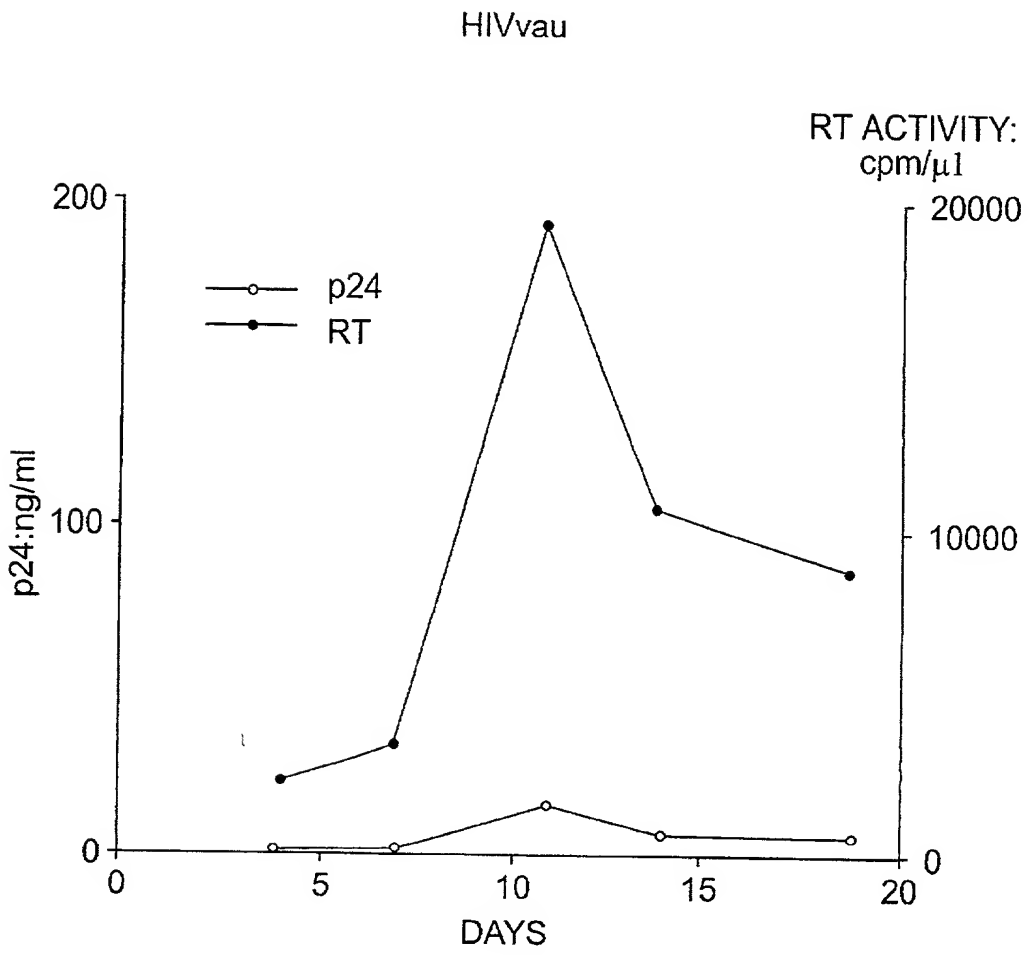
HIVvau
RT ACTIVITY:
cpm/µl
20000
10000
0
200
100
0
p24:ng/ml
p24
RT
0
5
10
15
20
DAYS

FIG. 1B

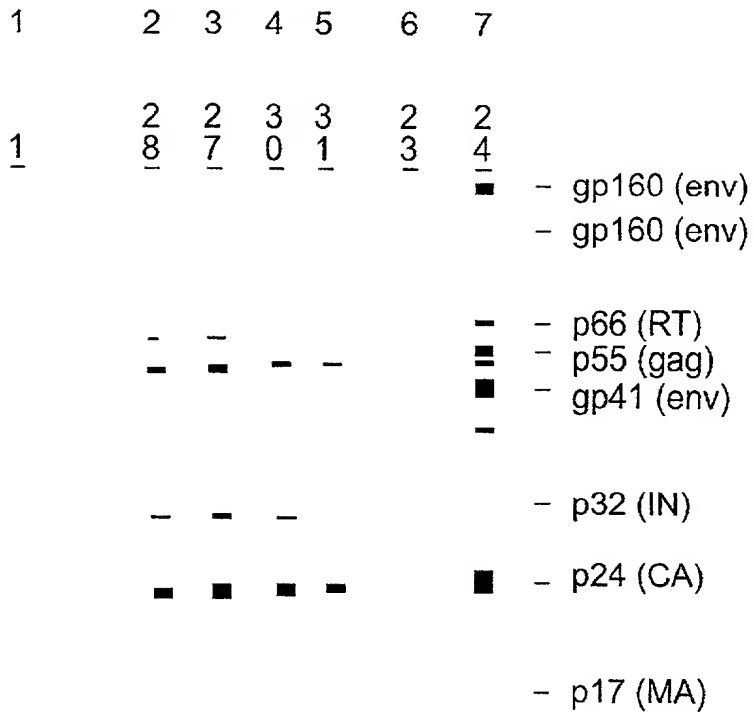
1
2
3
4
5
6
7
1
28
27
30
31
23
24
– gp160 (env)
– gp160 (env)
– p66 (RT)
– p55 (gag)
– gp41 (env)
– p32 (IN)
– p24 (CA)
– p17 (MA)

FIG. 2

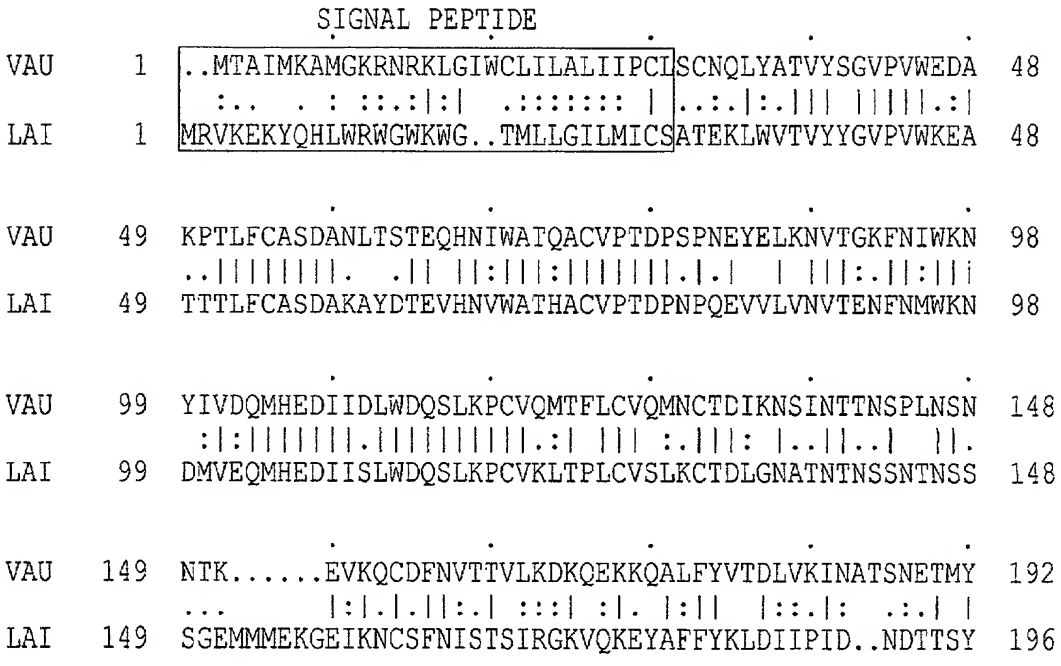

FIG. 3A

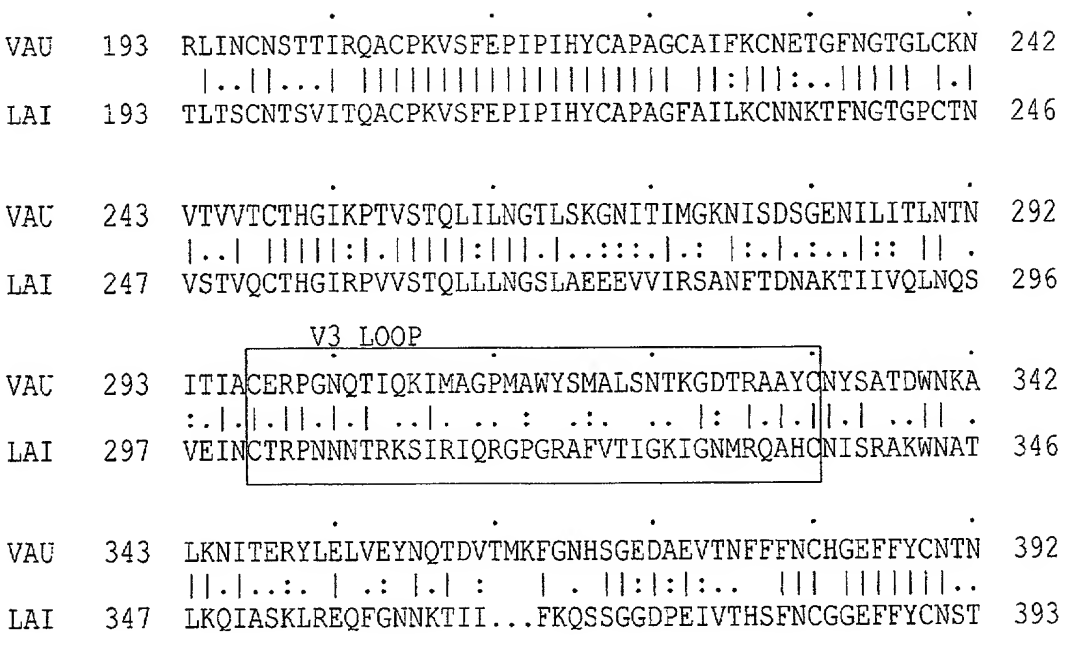
V3 LOOP
VAU 193 RLINCNSTTIRQACPKVSFEPIPIHYCAPAGCAIFKCNETGFNGTGLCKN 242
LAI 193 TLTSCNTSVITQACPKVSFEPIPIHYCAPAGFAILKCNNKTFNGTGPCTN 246
VAU 243 VTVVTCTHGIKPTVSTQLILNGTLSKGNITIMGKNISDSGENILITLNTN 292
LAI 247 VSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSANFTDNAKTIIVQLNQS 296
VAU 293 ITIACERPGNQTIQKIMAGPMAWYSMALSNTKGDTRAAYCNYSATDWNKA 342
LAI 297 VEINCTRPNNNTRKSIRIQRGPGRAFVTIGKIGNMRQAHCNISRAKWNAT 346
VAU 343 LKNITERYLELVEYNQTDVTMKFGNHSGEDAEVTNFFFNCHGEFFYCNTN 392
LAI 347 LKQIASKLREQFGNNKTII...FKQSSGGDPEIVTHSFNCGGEFFYCNST 393
VAU 393 RLFNHTFSCKKNMTNNKINCTNISNNSNGTQAI..PCRLRQVVRDWMRGG 440
LAI 394 QLFNSTWF.....NSTWSTEGSNNTEGSDTITLPCRIKQFINMWQEVG 436

FIG. 3B

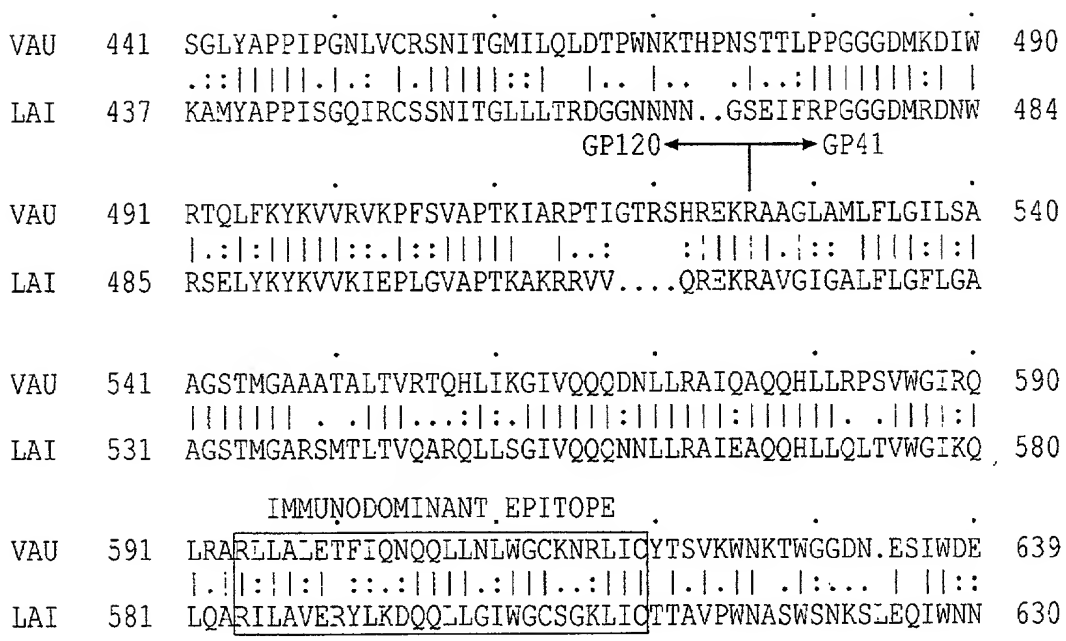
VAU 441 SGLYAPPIPGNLVCRSNITGMILQLDTPWNKTHPNSTTLPPGGGDMKDIW 490
LAI 437 KAMYAPPISGQIRCSSNITGLLLTRDGGNNNN..GSEIFRPGGGDMRDNW 484
GP120
GP41
VAU 491 RTQLFKYKVVRVKPFSVAPTKIARPTIGTRSHREKRAAGLAMLFLGILSA 540
LAI 485 RSELYKYKVVKIEPLGVAPTKAKRRVV....QREKRAVGIGALFLGFLGA
VAU 541 AGSTMGAAATALTVRTQHLIKGIVQQQDNLLRAIQAQQHLLRPSVWGIRQ 590
LAI 531 AGSTMGARSMTLTVQARQLLSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQ 580
IMMUNODOMINANT EPITOPE
VAU 591 LRARLLALETFIQNQQLLNLWGCKNRLICYTSVKWNKTWGGDN.ESIWDE 639
LAI 581 LQARILAVERYLKDQQLLGIWGCSGKLICTTAVPWNASWSNKSLEQIWNN 630

FIG. 3C

```
VAU   640  LTWQQWDQQINNVSSFIYEKIQEAQEQQEKNEKELLELDEWASIWNWLDI  689
           :|| :||.:||| .|:|.. |:|.|:||||||.||||||.|||:|||::|
LAI   631  MTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFNI  680

VAU   690  TKWLWYIKIAIIIVGALIGVRVVMIVLNLVKNIRQGYQPLSLQIPIQQQA  739
           |.|||||| |:|||:|:|:|:|: ||.:|..:|||| |||:|..:.~.
LAI   681  TNWLWYIKIFIMIVGGLVGLRIVFAVLSIVNRVRQGYSPLSFQTHLPTPR  730

VAU   740  EVGTPGGTGEGGGDEDRRRWTPLPQGFLHLLYTDLRTIILWIYHLLSNLA  789
           :.: |:|.:|:||: || |...|..| | |::.|||.:.|: || |.:|
LAI   731  GPDRPEGIEEEGGERDRDRSIRLVNGSLALIWDDLRSLCLFSYHRLRDLL  780

VAU   790  SEIQKLIRHLGLGLWIIGQRTIEACRLFKAIIQYWLQELQTSATNLLDTV  839
             : :::        ::|.|. || :.:..::||| |||..||..||:..
LAI   781  LIVTRIVE.......LLGRRGWEALKYWWNLLQYWSQELKNSAVSLLNAT  823

VAU   840  AVAVANWTDSTILGIQSIGRGILNIPRRIRQGLERLLL  877
           |:|||: ||..| .:|: .|:| :||||||||||:||
LAI   824  AIAVAEGTDRVIEVVQGACRAIRHIPRRIRQGLERILL  861
```

FIG. 3D

```
HIV-1lai        RILAVERYLKDQQLLGIWGCSGKLIC
HIV-1Z321       ------------------------I--
HIV-1eli        ------------------------H--
HIV-1JRCSF      -V------------M-----------
HIV-1WMJ        -V-------R----------------
HIV-1NDK        -V-------R------------RH--
HIV-1mal        -V-------Q--R---M------H--
SIVCPZGAB       -L-------Q---I--L------AV-

vau             -L--L-TFIQN----NL---KNR---
mvp5180         -LQ-L-TLIQN--R-NL---K-----
ant70           -L--L-TL-QN----SL---K---V-

HIV-2rod        -VT-I-K--Q--AR-NS---AFRQV-
HIV-2D194       -VT-I-K-----AQ-NS---AFRQV-
```

FIG. 4

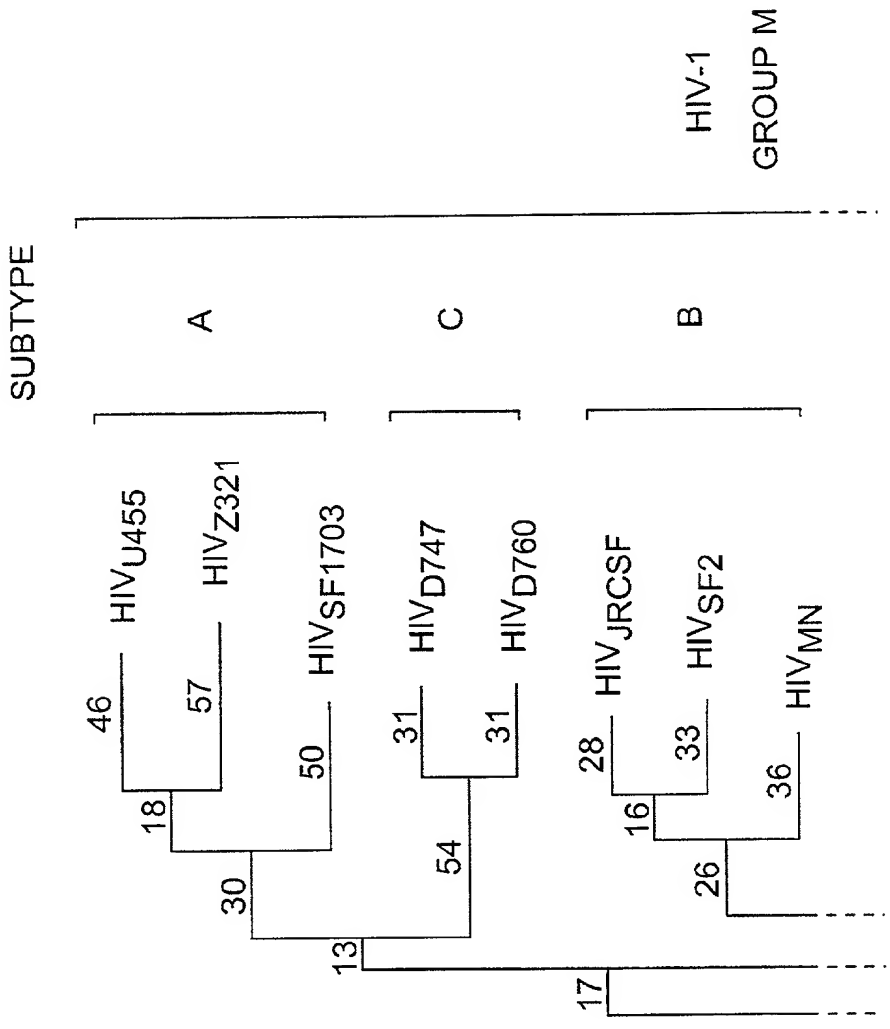
HIV-1
GROUP M
SUBTYPE
A
C
B
HIV$_{U455}$
HIV$_{Z321}$
HIV$_{SF1703}$
HIV$_{D747}$
HIV$_{D760}$
HIV$_{JRCSF}$
HIV$_{SF2}$
HIV$_{MN}$
46
57
50
31
31
28
33
36
18
54
16
26
30
13
17

FIG. 5A

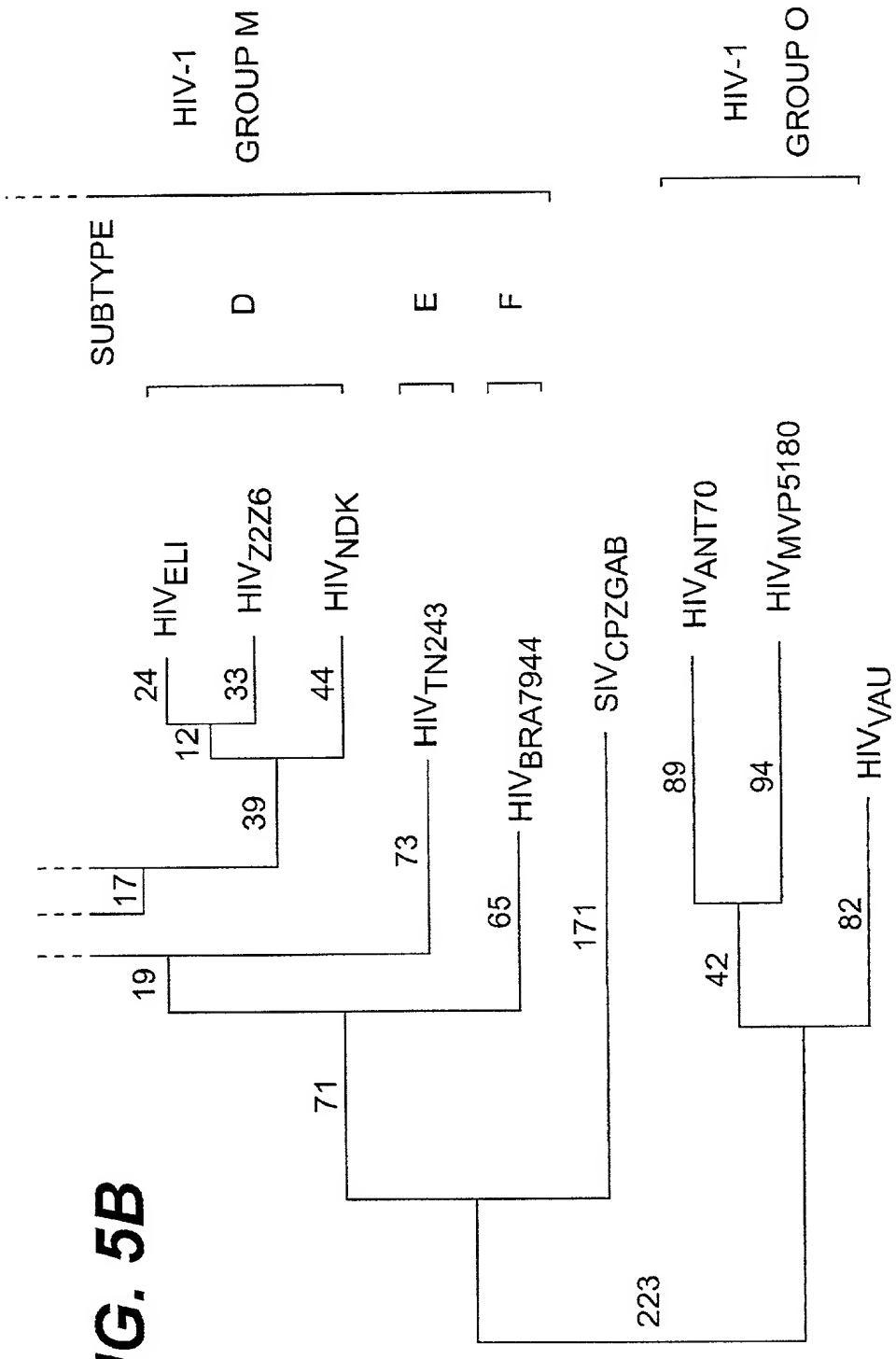
HIV-1
GROUP M
HIV-1
GROUP O
SUBTYPE
D
E
F
HIV$_{ELI}$
HIV$_{Z2Z6}$
HIV$_{NDK}$
HIV$_{TN243}$
HIV$_{BRA7944}$
SIV$_{CPZGAB}$
HIV$_{ANT70}$
HIV$_{MVP5180}$
HIV$_{VAU}$
24
33
44
12
39
17
73
65
19
71
171
89
94
42
82
223

FIG. 5B

DNA SEQUENCE 2631 B.P. ATGACAGCGATT ... CGACTCCTGTTA LINEAR

```
      |  10     |  20     |  30     |  40     |  50     |  60     |  70     |  80     |  90     | 100
    1 ATGACAGCGA TTATGAAAGC AATGGGGAAG AGGAACAGGA AGTTAGGGAT CTGGTGCTTG ATTTTGGCTT TGATAATCCC ATGTTTGAGC TGTAACCAAC
  101 TATATGCCAC AGTCTATTCT GGGGTACCTG TATGGGAAGA TGCAAAACCA ACATTGTTCT GTGCTTCAGA TGCTAACTTG ACAAGCACTG AACAGCATAA
  201 TATTTGGGCA ACACAAGCCT GTGTTCCCAC AGACCCCAGT CCAAATGAAT ATGAGCTAAA AAATGTGACA GGTAAATTCA ATATATGGAA AAATTATATA
  301 GTAGACCAAA TGCACGAAGA CATTATAGAT TTGTGGGACC AGAGTTTAAA ACCTTGTGTT CAAATGACTT TCTTGTGTGT ACAAATGAAT TGTACAGATA
  401 TCAAAAATAG TATTAATACC ACAAACAGTC CCTTAAACTC AAACAATACA AAAGAGGTGA AACAGTGTGA CTTTAATGTA ACTACAGTGC TCAAAGACAA
  501 ACAGGAGAAA AAACAGGCTC TATTCTATGT GACAGATTTG GTTAAGATTA ACGCCACATC AAATGAAACA ATGTATAGAT TAATTAATTG TAACTCCACA
  601 ACCATCAGGC AGGCCTGTCC AAAGGTATCT TTTGAGCCCA TTCCCATACA CTATTGTGCT CCAGCGGGAT GTGCCATCTT TAAGTGTAAT GAAACAGGAT
  701 TTAATGGAAC AGGTCTCTGT AAAAACGTTA CAGTAGTTAC TTGTACACAT GGCATCAAAC CAACAGTAAG TACCCAACTA ATACTAAATG GGACACTCTC
  801 TAAAGGAAAT ATAACAATCA TGGGAAAGAA TATTTCAGAC AGTGGGGAGA ACATCCTAAT AACCCTAAAT ACTAATATAA CAATAGCATG TGAGAGACCA
  901 GGAAATCAGA CAATACAAAA GATAATGGCA GGTCCAATGG CTTGGTACAG CATGGCCCTT AGTAATACAA AGGGGGATAC AAGGGCAGCT TATTGTAATT
 1001 ATAGTGCCAC TGACTGGAAC AAAGCCTTAA AAAACATAAC TGAAAGATAT TTAGAACTTG TAGAATATAA TCAAACTGAT GTTACCATGA AATTCGGTAA
 1101 TCACAGTGGT GAAGATGCAG AAGTAACAAA TTTCTTTTTT AACTGTCATG GAGAATTCTT TTATTGTAAC ACAAATCGGC TGTTTAATCA TACCTTTTCC
 1201 TGCAAGAAGA ATATGACCAA TAACAAGATC AATTGTACTA ATATTAGCAA TAATAGCAAT GGCACTCAGG CAATACCTTG CAGGTTGAGA CAAGTAGTAA
 1301 GGGACTCGAT GAGGGGAGGA TCGGGACTTT ATGCACCTCC CATCCCAGGA AACCTAGTAT GCAGGTCAAA CATAACTGGA ATGATTCTAC AATTGGACAC
 1401 GCCATGGAAT AAAACACATC CTAACAGCAC CACCCTTAGA CCAGGAGGGG GAGATATGAA AGATATATGG AGAACTCAAT TGTTCAAATA TAAAGTAGTA
 1501 AGAGTAAAAC CTTTTAGTGT AGCACCAACA AAAATTGCAA GGCCAACTAT AGGAACTAGA TCTCATAGAG AGAAAAGAGC AGCAGGTTTG GCAATGCTAT
 1601 TCTTGGGGAT TCTAAGTGCA GCAGGAAGCA CTATGGGCGC AGCGGCAACA GCGCTGACGG TACGGACCCA GCATCTGATA AAGGGTATAG TGCAACAGCA
 1701 GGATAACCTG CTAAGAGCAA TACAGGCCCA GCAACACTTG CTGAGGCCAT CTGTATGGGG TATTAGACAA CTCCGAGCTC GCCTGCTAGC CTTAGAAACC
 1801 TTTATACAGA ATCAGCAACT CCTTAACCTG TGGGGCTGCA AGAATAGACT AATCTGCTAC ACATCAGTAA AGTGGAATAA AACATGGGGA GGAGATAATG
 1901 AATCAATTTG GGATGAGTTA ACATGGCAGC AGTGGGATCA ACAGATAAAC AACGTAAGCT CCTTCATATA TGAAAAAATA CAAGAGGCAC AAGAACAACA
 2001 GGAGAAAAAT GAGAAAGAAT TGCTGGAGTT AGATGAATGG GCCTCTATTT GGAATTGGCT TGACATAACT AAATGGTTGT GGTATATAAA AATAGCTATA
 2101 ATCATAGTAG GAGCACTAAT AGGTGTAAGA GTAGTTATGA TAGTACTTAA TCTAGTAAAG AACATTAGGC AGGGATATCA ACCCCTCTCG TTACAGATCC
 2201 CCATCCAACA ACAAGCGGAA GTAGGAACGC CAGGAGGAAC AGGAGAAGGA GGTGGAGACG AAGACAGGCG CAGGTGGACT CCATTGCCGC AAGGGTTCTT
 2301 GCATCTGTTG TACACGGACC TCAGGACAAT AATCTTGTGG ATTTACCACC TCTTGAGCAA CTTAGCCTCA GAGATCCAGA AGTTGATCAG ACACCTGGGA
 2401 CTTGGACTAT GGATCATAGG GCAGAGGACA ATTGAAGCTT GCAGACTCTT TAAAGCTATA ATACAATACT GGCTACAAGA ATTGCAAACT AGTGCTACAA
 2501 ATCTACTAGA TACTGTTGCA GTGGCAGTTG CTAATTGGAC TGACAGCACA ATCTTAGGCA TACAAAGCAT AGGGAGAGGG ATTCTTAACA TACCAAGAAG
 2601 GATTAGACAG GGCCTTGAAC GACTCCTGTT A
      |  10     |  20     |  30     |  40     |  50     |  60     |  70     |  80     |  90     | 100
```

FIG. 6

```
      |   10     |   20     |   30     |   40     |   50     |   60     |   70     |   80
  1 GCAGAGACAG GACAGGAAAC TGCCTACTTC CTGTTAAAAT TAGCAGCAAG ATGGCCTATT AAAATACTAC ATACAGACAA
 81 TGGGCCTAAC TTTACAAGTG CAGCCATGAA AGCTGCATGT TGGTGGACAA ACATACAACA TGAGTTTGGA ATACCATACA
161 ATCCACAAAG TCAAGGAGTA GTAGAAGCCA TGAACAAGGA ATTAAAATCA ATCATACAGG TGAGGGACCA AGCAGAGCAC
241 TTAAGGACAG CAGTACAAAT GGCAGTATTT GTTCACAATT TTAAAAGAAA AGGGGGGATT GGGGGGTACA CTGCAGGAGA
321 GAGATTAATA GACATATTAG CATCACAAAT ACAAACAACA GAACTACAAA AACAAATTTT AAAAATTCAA AATTTTCGGG
401 TCTATTACAG AGACAGCAGA GACCCTATTT GGAAAGGACC GGCACAGCTC CTG
      |   10     |   20     |   30     |   40     |   50     |   60     |   70     |   80
```

FIG. 7

GAG REGION

```
                         #      # #    #        #        #      #
DUR    qgqmvhqalsprtlnawvkaveekafnpeiipmfmalsegavpydinvmlnaigghqgal
ANT    --------i--------------------------------is----t------------
MVP    --------i--------------------------------------t------------
LAI    --------i------v----------s--v----s------t-q-l-t---tv----a-m
MAL     ---i---i------vi---------s--v----s------t-q-l-m---iv----a-m
CPZ     -------i------v----------s--v----s------l-q-v-t----v------m
```

FIG. 8A

```
                                  #   #    #   #          #    #

DUR     qvlkevindeaadwdrahpqqagplppgqireptgsdiagttstqqeqilwttragnpip
ANT     --------e--ve---t--ppv----------------------------h----pnq---
MVP     --------e---e---t--pam----------------------------i----gans--
LAI     -m---t--e---e---v--vh---ia---m---r----------l----g-m-nnp_---
MAL     -m--dt--e-------v--vh---i----m---r----------l----g-m-_snp---
CPZ     --------e---e---l--th---ia---l---r----------l----g---_-np---
                            |||                                 |||
```

FIG. 8B

```
                #  #       #

DUR     vgdiyrkwivlglnkmvkmyspvsildirqgpkepfrdyvdrfyktlrae
ANT     ---------------------------k--------------------
MVP     --------------------------------------------------
LAI     --e--kr--i-----i-r----t-------------------------
MAL     -----kr--i-----i-r-----------------------f------
CPZ     ---v--r-vi-----v-r--c---------------------------
```

HIV1-M / HIV1-O DISCRIMINATING POSITIONS : #

HYPERVARIABLE REGIONS : ||||

FIG. 8C

REGION OF THE V3 LOOP OF GP120

```
----YK---QRTG-___---Q-LY-__THR-I-DI_-----        MAD
CTRPNNNTRKSIRIQRGPGRAFVT__IGK_IGNM__RQAHC        LAI
---------NR-S-_------H--__TKQ-I-DI__-----        OYI
-A--YQ---QRTP-__-L-QSLY-__TR_SRSII__G----        ELI
----G-----RGIHF__---QALY-__T-_-V-DI__-R-Y-       MAL
-S--Y-TRKNIRRYSI-S-QAFYV__T---I-DI__-Q---        455
-H--G----GE__VQI---MTFYN__-ENVV-DT__-S-Y-        CPZ


-E--QI_DIQE__MRI--_M-WYSMG--GTA--S_S---Y-        ANT
-I-EGIAEVQD-YT__--_MRWRSM_TLIRSNNT_S-V-Y-        MVP
-E--G-QTIQK-MA__--_M-WYSMALSNTK-DT__-A-Y-        VAU
-V--G--SV-E-K-__--_M-WYSMQ-EREGKGANS-T-F-        DUR

C*R********I****GP_M*W*SM**********SR*A*C HIV-O CONSENSUS
CVRPGNNSVKEIKI__GP_MAWYSMQIEREGKGANSRTAFC        DUR
```

FIG. 9A

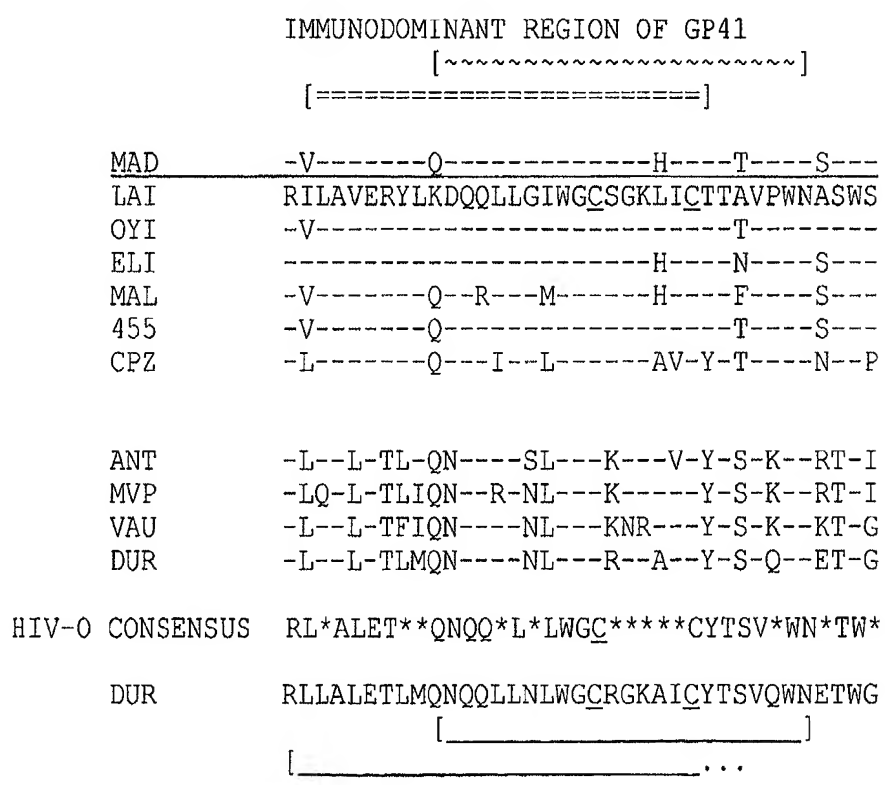
IMMUNODOMINANT REGION OF GP41
MAD -V-------Q----------H----T----S---
LAI RILAVERYLKDQQLLGIWGCSGKLICTTAVPWNASWS
OYI -V----------T--------
ELI ----------H----N----S---
MAL -V-------Q--R---M------H----F----S---
455 -V-------Q----------T----S---
CPZ -L-------Q---I--L------AV-Y-T----N--P
ANT -L--L-TL-QN----SL---K---V-Y-S-K--RT-I
MVP -LQ-L-TLIQN--R-NL---K-----Y-S-K--RT-I
VAU -L--L-TFIQN----NL---KNR---Y-S-K--KT-G
DUR -L--L-TLMQN----NL---R--A--Y-S-Q--ET-G
HIV-0 CONSENSUS RL*ALET**QNQQ*L*LWGC*****CYTSV*WN*TW*
DUR RLLALETLMQNQQLLNLWGCRGKAICYTSVQWNETWG

FIG. 9B

CAGGGACAAATGGTACATCAGGCCATCTCCCCCAGAACTTTATATGTATGGGTAAAGGCA
GTAGAAGAAAAGGCCTTTAACCCTGAAATTATCCCTATGTTTATGGCACTATCAGAAGGA
GCTGTTCCCTATGATATCAATGTTATGCTAAATGCCATAGGAGGACACCAAGGGGCTTTA
CAAGTATTAAAAGAAGTAATCAATGATGAAGCAGCAGACTGGGATAGAGCTCACCCACAA
CAGGCAGGGCCGTTACCACCAGGGCAGATAAGGGAACCAACAGGAAGTGACATTGCTGGA
ACAACTAGCACACAGCAAGAGCAAATTCTCTGGACTACTAGGGCAGGTAACCCTATCCCA
GTTGGAGACATCTATAGGAAATGGATAGTGTTGGGTCTAAACAAAATGGTAAAAATGTAT
AGTCCAGTGAGCATCTTAGATATTAGGCAGGGACCAAAAGAACCATTTAGAGATTATGTA
GACAGGTTCTACAAAACATTAAGAGCTGAGCAG

GAG REGION OF HIV1-O DUR STRAIN: 513 BASE PAIRS

= SEQ ID N° 9

QGQMVHQALSPRTLNAWVKAVEEKAFNPEIIPMFMALSEGAVPYDINVMLNAIGGHQGAL
QVLKEVINDEAADWDRAHPQQAGPLPPGQIREPTGSDIAGTTSTQQEQILWTTRAGNPIP
VGDIYRKWIVLGLNKMVKMYSPVSILDIRQGPKEPFRDYVDRFYKTLRAEQ

GAG REGION OF HIV1-O VIRUS DUR STRAIN: 171 AMINO ACIDS

FIG. 10

ATTCCAATACACTATTGTGCTCCAGCAGGATATGCTATCTTTAAATGCAACAACGAGGAG
TTTACTGGAAAAGGCCCATGTAACAACATTTCAGTAGTTACCTGTACACAGGGTATCAAG
CCAACAGTAAGCACTCATCTAATATTCAATGGGACAATCTCTGAAAGAAAAATAAGAATT
ATGGGAAAGAACATCTCGAGCAACTCAGGTAATATCCTAGTGACCCTAAATTCTACTATA
AACATGACCTGTGTGAGGCCAGGAAATAATTCAGTACAGGAGATAAAAATAGGTCCAATG
GCTTGGTACAGTATGCAAATTGAGCGAGAGGGAAAAGGAGCAAATTCAAGAACAGCTTTT
TGTACCTATAATGCCACGGACTGGAGAAAAACCTTGCAAGGGATAGCTGAAAGGTATTTA
GAACTTGTAAATAAAACAAGTCCGACTGAAATAATGTTCAATAAAAGCAATGGTGGAGAT
GCACAAATAACCCGTTTGCATTTTAACTGTCATGGAGAATTCTTT

V3 LOOP OF GP120 DUR STRAIN: 525 BASE PAIRS

= SEQ ID N° 10

IPIHYCAPAGYAIFKCNNEEFTGKGPCNNISVVTCTQGIKPTVSTHLIFNGTISERKIRI
MGKNISSNSGNILVTLNSTINMTCVRPGNNSVQEIKIGPMAWYSMQIEREGKGANSRTAF
CTYNATDWRKTLQGIAERYLELVNKTSPTEIMFNKSNGGDAEITRLHFNSCGEFF

V3 LOOP OF GP120 DUR STRAIN: 175 AMINO ACIDS

FIG. 11

ATAGTGCAACAGCAGGACAACCTGCTGAGAGCAATACAGGCCCAGCAACATCTGCTGAGG
TTATCTGTATGGGGTATTAGACAACTCCGAGCTCGCCTGCTAGCCTTAGAAACCCTTATG
CAGAATCAGCAACTCCTAAACCTGTGGGGTTGTAGAGGAAAAGCAATCTGCTACACATCA
GTACAATGGAATGAAACATGGGGAGGAAATGACTCAATTTGGGACAGGTTAACATGGCAG
CAATGGGATCAACAGATAGCCAATGTAAGCTCTTTTATATATGACAAAATACAAGAAGCA
CAAGAACAACAA

DNA SEQUENCE OF THE IMMUNODOMINANT REGION OF GP41 OF HIV1-O DUR: 312 BASE PAIRS

= SEQ ID N° 11

IVQQQDNLLRAIQAQQHLLRLSVWGIRQLRARLLALETLMQNQQLLNLWGCRGKAICYTS
VQWNETWGGNDSIWDRLTWQQWDQQIANVSSFTYDKIQEAQEQQ

DNA SEQUENCE OF THE IMMUNODOMINANT REGION OF GP41 OF HIV1-O DUR - PREDICTED PROTEIN: 104 AMINO ACIDS

FIG. 12

SPECIFIC PRIMERS OF THE HIV-O TYPE

DUR V3a	ATT-CCA-ATA-CAC-TAT-TGT-GCT-CCA-3′
DUR V3r	AAA-GAA-TTC-TCC-ATG-ACT-GTT-AAA-3′
DUR 41a	GGT-ATA-GTG-CAA-CAG-CAG-GAC-AAC-3′
DUR 41r	AGA-GGC-CCA-TTC-ATC-TAA-CTC-3′

FIG. 13A

POSITIONS OF THE PRIMERS:

IN HIV MVP5180	
dur V3a	6896 TO 6919
dur V3r	7400 TO 7423
dur 41a	7934 TO 7957
dur 41r	8292 TO 8302
IN HIV ANT70	
dur V3a	6896 TO 6920
dur V3r	7392 TO 7415
dur 41a	7917 TO 7940
dur 41r	8256 TO 8276
IN HIV1 VAU	
dur V3a	640 TO 663
dur V3r	1138 TO 1161
dur 41a	1684 TO 1707
dur 41r	2026 TO 2046

FIG. 13B

V3	
HIV1-M CONSENSUS	NEGATIVE
HIV1-M MAL (AFRICAN)	NEGATIVE
HIV1-M CTV-CPZ (CHIMPANZEE)	NEGATIVE
HIV1-O MVP5180	NEGATIVE
HIV1-O ANT70	POSITIVE

GP41	
HIV1-M CONSENSUS:	
-PASTEUR STANDARD	NEGATIVE
-INNOGENETICS RIGHT-EXTENDED	WEAK POSITIVE
HIV1-O MVP5180:	
-INNOGENETICS	NEGATIVE
-BEHRING LEFT-EXTENDED	POSITIVE
HIV1-O VAU	POSITIVE

FIG. 14

NUCLEOTIDE COMPARISONS

EXPRESSED AS PERCENTAGE DIFFERENCE

GP41 (OUT OF 330 BASES)

LAI	0						
MAL	11	0					
CPZ	33	31	0				
MVP5180	39	38	38	0			
ANT70	36	39	37	15	0		
VAU	39	38	38	14	14	0	
DUR	38	36	37	13	15	11	0
	LAI	MAL	CPZ	MVP 5180	ANT 70	VAU	DUR

FIG. 15A

V3 (OUT OF 558 BASES)

LAI	0						
MAL	19	0					
CPZ	37	34	0				
MVP5180	46	43	45	0			
ANT70	45	44	43	23	0		
VAU	44	41	41	24	24	0	
DUR	46	43	42	25	22	24	0
	LAI	MAL	CPZ	MVP 5180	ANT 70	VAU	DUR

GAG (OUT OF 520 BASES)

LAI	0					
MAL	9	0				
CPZ	21	25	0			
MVP5180	24	26	25	0		
ANT70	25	25	24	10	0	
DUR	25	26	25	9	10	0
	LAI	MAL	CPZ	MVP 5180	ANT 70	DUR

FIG. 15B

PROTEIN COMPARISONS

EXPRESSED AS PERCENTAGE DIFFERENCE

GP41 (OUT OF 109 AMINO ACIDS)

LAI	0						
MAL	17	0					
CPZ	33	28	0				
MVP5180	42	40	41	0			
ANT70	42	45	39	22	0		
VAU	44	47	45	19	21	0	
DUR	44	42	39	17	17	14	0
	LAI	MAL	CPZ	MVP 5180	ANT 70	VAU	DUR

FIG. 16A

V3 (OUT OF 186 AMINO ACIDS)

LAI	0						
MAL	31	0					
CPZ	46	39	0				
MVP5180	55	50	59	0			
ANT70	55	50	55	36	0		
VAU	55	51	55	39	36	0	
DUR	56	51	56	39	35	42	0
	LAI	MAL	CPZ	MVP 5180	ANT 70	VAU	DUR

GAG (OUT OF 174 AMINO ACIDS)

LAI	0						
MAL	6	0					
CPZ	11	14	0				
MVP5180	21	23	18	0			
ANT70	21	24	19	6	0		
DUR	22	22	19	7	9	0	
	LAI	MAL	CPZ	MVP 5180	ANT 70	DUR	

FIG. 16B

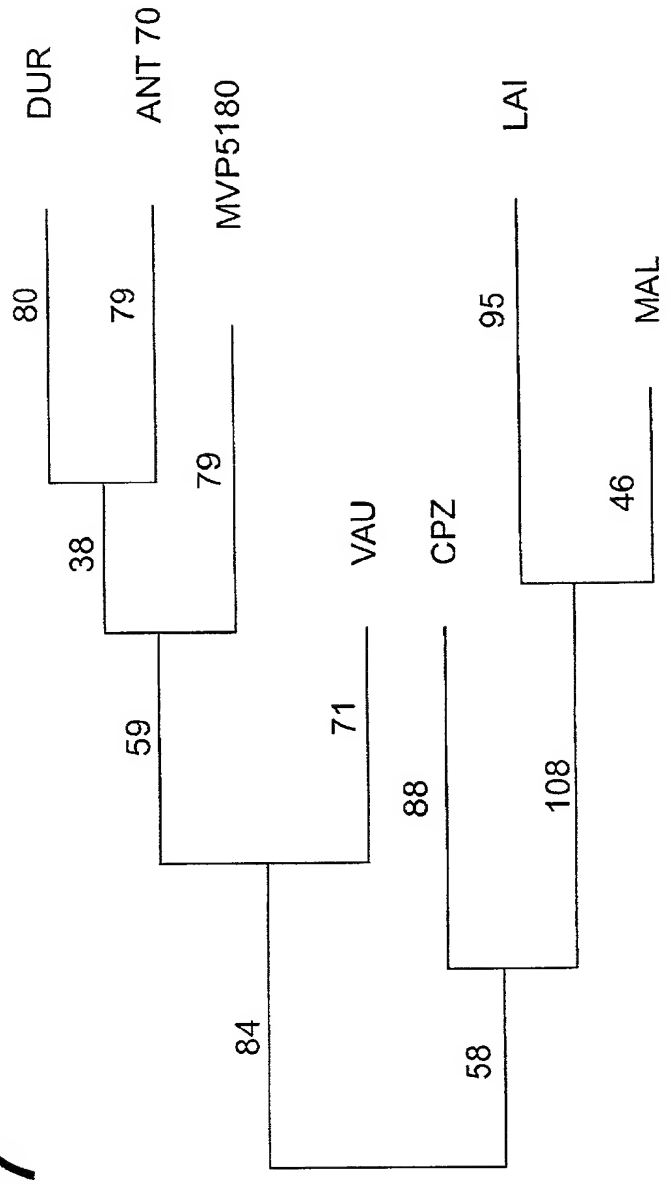

FIG. 17

PHYLOGENETIC TREE OF V3
(TREEALIGN PROGRAM, J. HEIN, ON BISANCE, CITI2)
(OUT OF 171 AMINO ACIDS)

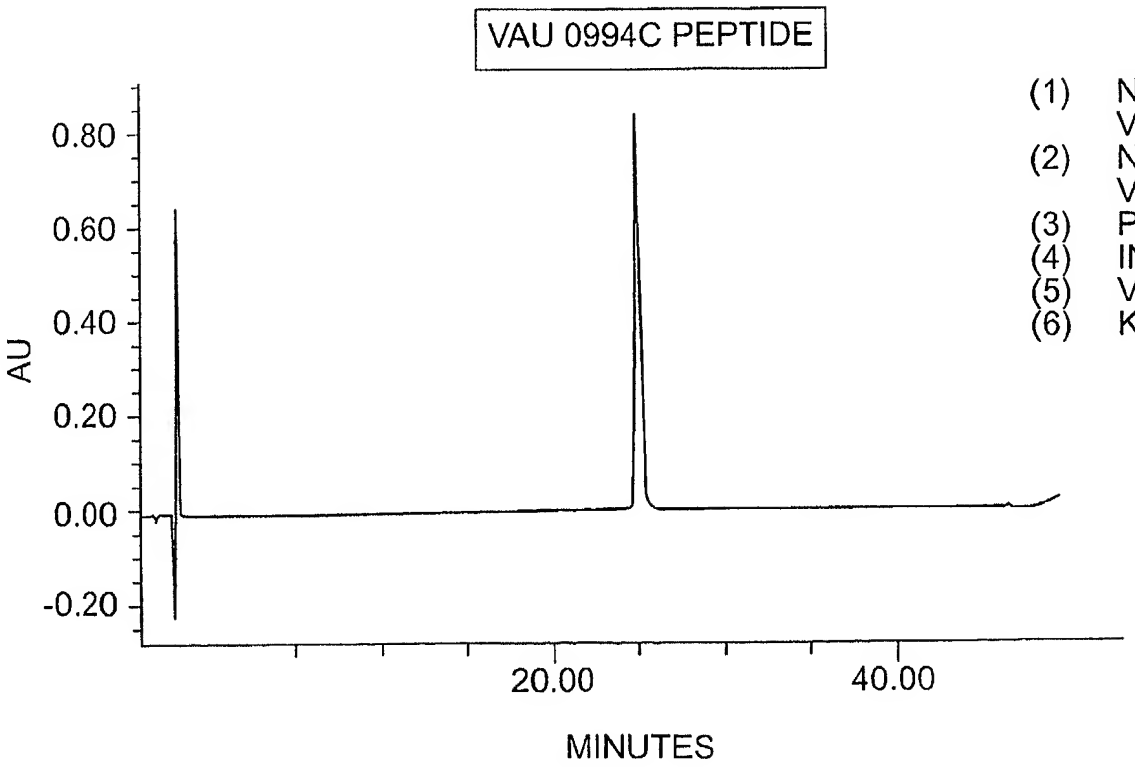
VAU 0994C PEPTIDE
0.80
0.60
0.40
0.20
0.00
-0.20
AU
20.00
40.00
MINUTES
(1) NAME OF THE SAMPLE: Vau7101550
(2) NAME OF THE METHOD: VauRepMel 3
(3) PROCESS: FvauPrcMel 3D
(4) INJECTION TIME: 45.0 MIN
(5) VOLUME: 50.00ml
(6) KIT: : MSHV15_5030mlml

FIG. 18A

CHROMATOGRAPHIC RESULTS

RETENTION TIME (MIN)	INITIAL TIME (MIN)	FINAL TIME (MIN)	% SURFACE AREA	INT TYPE
24.482	23.898	24.932	100.00	BB

FIG. 18B

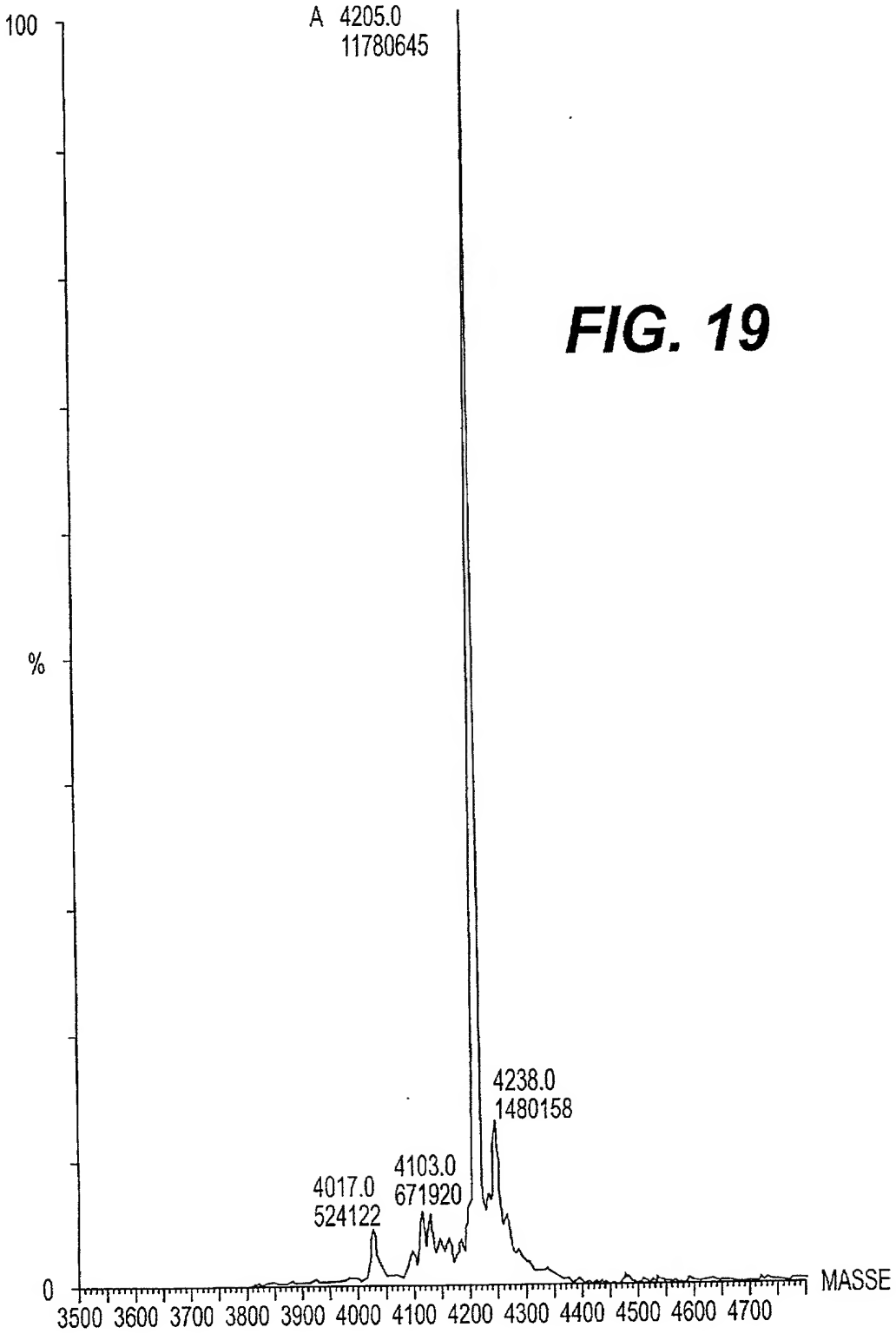
100
A 4205.0
11780645
%
4238.0
1480158
4103.0
671920
4017.0
524122
0
MASSE
3500 3600 3700 3800 3900 4000 4100 4200 4300 4400 4500 4600 4700

FIG. 19

VAU PEPTIDE

REF: F VAU 0994 C 710

SEQUENCE: RLLALETFIQNQQLLNLWGCKNRLICYTSVKWNKT

LENGTH: 35

MOLECULAR WEIGHT: 4210

ANALYTICAL CONTROLS: > 95% BY HPLC AND MASS SPECTROMETRY

FIG. 20

STUDY OF THE IMMUNOREACTIVITY OF THE PEPTIDE MIMICKING THE IMMUNODOMINANT EPITOPE OF THE GP160 VAU SEQUENCE (SUBTYPE O)

VS=0.1 RATIO	VAU PEPTIDE 2µg/ml
HIV1 SERA (PANEL BBI)	
BO1 NO° 12	0.80
BO1 NO° 13	0.40
BO1 NO° 15	0.80
PRB911K6	0.20
HIV1 SERA (ROMANIA) STAGE 3/4	
3989	9.50
5116	8.60
HIV1 SERA (PANEL AFM) SUBTYPE O	
MAA	>30
LOB	>30
HAM	>30
DUR	12.50
HIV1 SERUM (REIMS) SUB TYPE M?	
MAD	0.20

FIG. 21A

SUSPICION OF SUBTYPE O (YAOUNDE PASTEUR CENTER)	
	0.60
3372	>30
3361	28.80
1507	28.70
3167	>30
2628	28.10
1060	0.60
4020	0.30
4783	0.30
5322	0.40
6661	0.50
5527	0.30
5863	25.00
5969	>30
6487	>30
6509	0.70
6782	>30
5453	27.30
3826	1.50
HIV2 SERA	
BERT	0.30
PAOL	4.50
RIV	15.80

INDIRECT EIA PROCEDURE; 3 X 30 MIN TYPE GENELAVIA MIXT

NEGATIVE SERA	
N=48 AVERAGE	0.022
DS	0.007
AVERAGE +12DS	0.107
VS	0.100

FIG. 21B

SUMMARY OF THE RESULTS OBTAINED ON THE AFRICAN SERA

	WB1 RESULTS									
	GP 160	GP 120	P 68	P 55	GP 41	P 40	P 34	P 25	P 18	
3361	++	+-	+				++	+	+	POS
1507	+	+-					+-	+-		POS
2628	+-		++	++		+	+	++	++	POS
3167	++	+	++	++	+	+	++	++	++	POS
3372	++	+-	+	++	+	+	++	++	+-	POS
5453	++	+-	+	+	+-	+	+	+	+	POS
5863	++	+-	+	+	+-	+-	+	+	+	POS
5969	+--		+	+		+	+	+		IND
6487	++		+	+			+	+-	+-	IND
6782	++		+	+	+	+	+	+		POS
950	+-		+-				+-	+-	+-	IND
1060	+-							+-	+	IND
5527										?
6509	++		+-				+--			IND
6661	+							+		IND
4020= SEMT	+							(+-)		IND
4783= 5322	+-							+-	+-	IND
3826								+-	+-	IND
MAD	++	+	+	+	+	+	+	+	+	POS
DUR										
MAA										
LOB	+		++	+-			++	++		IND
HAM			+				+	+		IND

=RATIO<1
=RATIO>2

FIG. 22A

	TEST DE SCREENING (RATIO : DO/VS)				
		GEM IND HIV1+2	ABBOTT SDW HIV1+2	MUREX SDW HIV1+2	
3361	0.10	18.00	0.56	0.72	1.40
1507	0.97	14.25	3.03	5.35	0.98
2628	0.70	18.00	4.84	1.71	1.34
3167	0.38	18.30	11.89	>6	0.88
3372	0.19	16.80	11.63	3.76	0.47
5453	2.50	>20			1.70
5863	2.30	>20			1.90
5969	2.30	15.20			2.25
6487	0.32	19.70			1.90
6782	0.07	13.40			2.95
950	1.20	6.00	5.76	>6	0.68
1060	0.60	18.00	0.46	1.25	0.67
5527	0.27	2.40			0.52
6509	0.32	>16			2.14
6661	8.10	10.10			1.54
4020= SEMT	0.23	6.30	1.03	4.98	4.12
4783=	0.19	8.10	0.41		0.55
5322		10.90			0.52
3826		3.93		1.64	0.72
MAD	-	+	+		
DUR		>8	2.00	0.80	
MAA					
LOB		>19	2.00	2.70	1.50
HAM		>19	1.80	7.80	2.70

FIG. 22B

	EIA PEPTIDES							
	CLONATEC RAP HIV1	39D6 HIV1B	FER HIV1B	39A HIV1B	VAU HIV1O	MVPP 5180 HIV1O	BNR 19 HIV1O	PEPTI-LAV1-2
3361	+				28.8	25.80	NT	
1507	DUBIOUS			8.4	28.7	28.80	1.7	
2628				3.6	28.1	19.30	1.2	
3167	+			>30	>30	>30	1.6	
3372	+-			2.8	>30	24.00	4.9	
5453	-	4.36	1.37	3.6	27.3		1.6	-
5863	-	1.42	0.40	1.4	25		0.5	-
5969	-	0.94	1.90	19.4	>30		0.6	+
6487	+	25.75	5.76	>30	>30		0.7	
6782	-	0.64	0.49	0.8	>30		10.3	-
950					0.6		0.4	
1060	DUBIOUS			1.9	0.6	0.40	0.6	
5527	-			4.5	0.3		0.2	+/-
6509	+			16.9	0.7		0.2	
6661	+			2.8	0.5		0.4	
4020=	?Ag-			1.15	0.3		0.5	
SEMT				1.2	0.7		0.9	
4783=				2.5	0.3		0.3	
5322				4	0.4		0.3	
3826				1.6	1.5		0.7	
MAD		0.66	2.72	26.7	0.2		>30	-
DUR		>30	>30	>30	12.5		0.2	
MAA				NT	>30		NT	
LOB		1.02	5.62	12.5	>30		>30	
HAM		0.73	0.41	13.3	>30		0.7	

FIG. 22C